REMARKS

In a final office action, claims 21-34 have been rejected under 35 U.S.C. §103. In response, Applicants have added new claims 35-45 and provide the herein remarks. Claims 35-45 are currently pending. Reconsideration is respectfully requested.

Support for New Claims

New claims 35-45 have been added that are directed to a process for preparing a reversible gel. Support for new claim 35 can be found in the application, for example, on page 12, lines 4-7. Support for new claim 42 can be found in the application, for example, in Table I, Example 3 and Example 8.

Rejections Under §103

Claims 21-26 have been rejected under §103 as being unpatentable over Seppala et al. in view of Hovenkamp-Hermelink et al., and Batelaan et al. The Examiner recognizes that Seppala does not teach (1) starches from a plant that has been genetically modified; (2) a process wherein the starch is hydrophobized via amidation; and (3) attaching the hydrophobic group in the presence of a surfactant. The Examiner contends that these deficiencies are taught by Hovenkamp et al. and Bathelaan et al.

In response, Applicants have cancelled claims 21-26 and added new claims 35-45. New claims 35-45 are directed to methods of preparing a reversible gel and increasing the associate behavior of a starch.

Seppala et al. merely disclose hydrophobized natural starches containing up to 100% amylopectin. Nowhere in Seppala et al. is there any disclosure or suggestion that a hydrophobized natural starch containing up to 100% amylopectin (i.e. waxy corn) could be prepared to form a reversible gel, or have increased associative behavior.

Hovenkamp-Hermelink et al. disclose amylose-free starch from mutant potatoes. The amylose-free potato mutant of Hovenkamp et al. was isolated after screening 12,000 minitubers.

Nowhere in Hovenkamp is there any disclosure or suggestion to prepare a reversible gel or increase the associative behavior of a starch using an amylose-free potato mutant.. Importantly, there is no disclosure or suggestion that the amylose-free potato mutant of Hovenkamp et al. would possess the unexpected properties concerning the ability to form a reversible gel, and associative behavior, that Applicants have discovered.

Batelaan et al. disclose methods for amidation of a material having at least one carboxyl-containing polysaccharide (see page 3, line 31 to page 4, line 1). The carboxyl-containing polysaccharide includes carboxyl methyl starch. However, nowhere is there any disclosure or suggestion to utilize root or tuber starches from modified plants according to the present invention in a process for preparing a reversible gel or to increase the associative behavior of a starch.

Furthermore, Batelaan discloses a process only suitable for polysaccharides, which posses very distinct properties and behaviors as compared to the amylopectin root or tuber starches in the present invention.

Discussion Of The Striking Differences Among Starches Of Different Botanical Origin

Attached are copies of three documents that support Applicants' position that various starches having up to 100% amylopectin do not share similar properties and/or behaviors.

Frederiksson et al., Carbohydrate Polymers, 45 (1998), 119-134 disclose a comparison of a number of properties of wheat, rye, barley, waxy barley (having close to

100% amylopectin), high amylose barley, waxy maize, potato, amylopectin potato and pea starch.

With regards to the following properties, significant differences are reported: granule diameter, lipid content, crystallinity, phosphate content and chain length distribution. Of particular interest is the difference in chain length distribution. Amylopectin potato starch (APS) (which is a root or tuber starch as claimed in the present invention), has the highest fraction of long amylopectin unit-chains (35% compared to 24% in waxy maize and 21% in waxy barley starch).

Frederiksson et al. teach that having a high fraction of long amylopectin unit-chains is important for amylopectin retrogradation. The uniqueness of amylopectin potato starch (APS) is nicely depicted in figure 8a of the article in which the cereal starches are separated from the tuber starches and the amylose containing starches are separated from the amylopectin containing starches. APS has a unique position, leading to specific and unexpected advantages.

In Yoo et al., Carbohydrate Polymers, 49 (2002), 307-314, a comparison is described of amylopectin from 23 different sources in terms of molecular weight, gyration radius and density. From this study it is apparent that amylopectin from waxy maize has four times higher molecular weight, slightly higher gyration radius and consequently more than three times higher density of the amylopectin molecule than amylopectin potato starch.

Jane et al. J. Appl. Glycoscience, 50 (2003), 167-172, discuss various aspects of amylopectin and its granule structure based on an overview of relevant literature. The difference in branch structures between A-(cereal) and B-(tuber) crystalline starches are summarized on pages 167-168 and in figure 1. The structural differences between the cereal and tuber starches leads to different behaviors in the Naegeli (partial acid) hydrolysis process.

Jane et al. also rephrase the molecular weight and density differences already disclosed by Yoo et al. and attempt to link these to different botanical origins of the starches.

In sum, it is Applicants position, which is supported by the above documents, that the behavior of starches of different botanical sources and having different amylopectin contents is unpredictable.

Therefore, although the Examiner contends that the attachment of a hydrophobic group to a starch in general is known, one cannot predict what kind of rheological behavior the product would show, or which reagents would provide the most beneficial product. Hence, it would not have been obvious to one of ordinary skill to use a root or tuber starch having an amylopectin content of at least 95 %wt. in the claimed processes.

The examples of the application illustrate highly unexpected and beneficial rheological properties of the hydrophobic root or tuber starches of the present invention. To the person skilled in the art there was no reasonable expectation of success in achieving such properties starting from Seppala, not even if supplemented by Hovenkamp and/or Bathelaan, or any other cited references,

In order establish a *prima facie* case of obviousness, one of the criteria to be met is that there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to combine the teachings of the references

Nowhere in Seppala et al. or Batelaan et al. is there any disclosure or suggestion to replace the natural starch of Seppala et al., or the polysaccharide of Batelaan et al., with root or tuber starches having at least 95 wt.% of amylopectin in a process for preparing a reversible gel or to increase the associative behavior of a starch product.

Applicants herein have put forth evidence to support the idea that natural starches and polysaccharides do not have similar properties as root or tuber starches having at least 95 wt.% of amylopectin. Therefore, they are not interchangeable.

Since root or tuber starches having at least 95 wt.% of amylopectin and polysaccharides, such as natural starches, do not have similar properties, it would **not** have been obvious to replace the natural starches of Seppala et al. or to replace the polysaccharide of Batelaan et al. with root or tuber starches having at least 95 wt.% of amylopectin.

Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejections of claims 21-26 under §103(a).

Claim 27 (a hydrophobized amylopectin starch product) has been rejected under 35 U.S.C. §103(a) as being unpatentable over Seppala et al., in view of Hovenkamp-Hermelink et al.

As mentioned above, Applicants have cancelled claims 21-34 and added new claims 35-45. New claims 35-45 are directed to methods of preparing a reversible gel and increasing the associate behavior of a starch. Thus, the rejection has been rendered moot.

Claim 28 (a method for thickening a starch) has been rejected under 35 U.S.C. §103(a) as being unpatentable over Seppala et al. in view of Hovenkamp-Hermelink et al and Bathelaan et al.

In response, claim 28 has been cancelled. Thus, the rejection has been rendered moot.

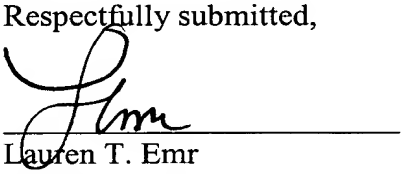
Claims 29-34 have been rejected under §103(a) as being unpatentable over the combination of Seppala et al., Hovenkamp-Hermelink et al. and Bathelaan et al., as applied to claims 21-28, and further in view of U.S. Patent No. 5,563,251 to Lachocki and U.S. Patent No. 5,977,348 to Harris. Applicants respectfully disagree.

Claims 29-34 differ from claims 21-28 in that the hydrophobization is limited to etherification and esterification, wherein the reagent comprises a halide, halohydrin, epoxide, glycidyl or quarternary ammonium.

In response, Applicants have cancelled claims 29-34 and added new claims 35-45 which are directed to a process for preparing a reversible gel and for increasing the associative behavior of a starch product.

In view of the foregoing amendments and remarks, applicants respectfully submit that the application is now in condition for allowance and is earnestly requested. If the Examiner believes that a discussion with Applicants' representative would be of assistance, he is invited to contact the undersigned.

Respectfully submitted,



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The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches

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Physico-chemical properties of starch from wheat, rye, barley (waxy, high-amylose and normal-amylose), waxy maize, pea and potato (normal-amylose and high-amylopectin) were studied. Emphasis was given to the amylose (total, apparent and lipid-complexed) and amylopectin characteristics as well as to the gelatinization and retrogradation properties measured using differential scanning calorimetry. The total amylose content varied from *ca.* 1% for waxy maize to 37% for high-amylose barley. The amylopectin characteristics were determined by high-performance size-exclusion chromatography after debranching with isoamylase. The weight-average degree of polymerization ($\overline{DP}_w$) was 26, 33 and 27 for the A-, B-, and C-type starches, respectively. In general, the potato starches exhibited the highest retrogradation enthalpies and the cereal starches the lowest, while the pea starch showed an intermediate retrogradation enthalpy. The data were analysed by principal component analysis (PCA). The $\overline{DP}_w$ showed positive correlation to the melting interval, the peak minimum, the offset temperatures of the retrogradation-related endotherm as well as to the gelatinization and retrogradation enthalpies. However, the high-amylose barley retrograded to a greater extent than the other cereal starches, despite low $\overline{DP}_w$ (24). The amylose content was negatively correlated to the onset and the peak minimum temperatures of gelatinization.

INTRODUCTION

Starch often contributes to the characteristic properties of foods, and is also added as a functional ingredient in many products. The demand for functionality may vary for different products and, in order to fulfil this demand, chemical modification of the starch is often needed. The botanical source is of great importance for the chemical and functional properties of a starch, but only in a few studies have starches originating from different starch-rich crops been compared using the same experimental methods (Orford et al., 1987; Kalichevsky et al., 1990; Shi and Seib, 1992; Ward et al., 1994). The relations between chemical composition and functional properties are therefore still, to a great degree, unknown.

The physical properties of starch are influenced by the amylose/amylopectin ratio. During gelatinization, the starch granules swell and form gel particles. In general, the swollen granules are enriched in amylopectin, while the linear amylose diffuses out of the swollen granules and makes up the continuous phase outside the granules (Hermansson and Svegmark, 1996). Waxy starches usually swell to a greater extent than their normal-amylose counterparts (Tester and Morrison, 1990a), and amylose is proposed to act as a restraint to swelling (Hermansson and Svegmark, 1996). Internal lipids in native cereal starches have been shown to have effects on the swelling and gelatinization properties of the granules (Morrison, 1995). The term retrogradation is used to describe the changes that occur upon cooling and storage of gelatinized starch. Short-term development of crystallinity in starch gels is attributed to the gelation and crystallization of the amylose fraction (Miles et al., 1985; Sievert and Würsch, 1993). Retrograded amylose or resis-

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Table 1. The different starch samples used, their X-ray diffraction pattern (A, B or C) and other characteristics

Starch type		X-ray diffraction pattern	Characteristics
Wheat	c.v. Holme	A[a]	Bread wheat
Rye	c.v. Motto	A[a]	Population rye
Barley	c.v. Golf	A[b]	Normal-amylose, covered
	Line 906129 waxy	A[b]	High-amylopectin, covered
	c.v. Glacier	A[b]	High-amylose, covered
Waxy maize	c.v. unknown	A[b]	High-amylopectin
Potato	c.v. Desiree	B[b]	Consumption potato
	cv. Prevalent	B[b]	Starch potato
	PAP	B[c]	High-amylopectin
Pea	c.v. Capella	C[c]	Smooth yellow pea

[a]Katz and van Itallie (1930)
[b]Zobel (1988)
[c]Analysed

tant starch type III (Englyst et al., 1992) is a physiologically important indigestible starch fraction. This fraction is heat stable and melts above 120°C (Sievert and Pomeranz, 1989). The long-term changes that occur during storage of starch gels have been attributed to the amylopectin fraction (Eliasson, 1985). The retrogradation behaviour of amylopectin has been related to the starch source (Orford et al., 1987; Kalichevsky et al., 1990; Shi and Seib, 1992; Ward et al., 1994) and concentration (Orford et al., 1987; Slade and Levine, 1987). Other factors, such as the storage temperature (Slade and Levine, 1987; Eliasson and Ljunger, 1988), amylopectin structure and other components present, are also of importance. Lipids (Eliasson and Gudmundsson, 1996) and short amylopectin unit-chains with degree of polymerization (DP) 6–9 (Shi and Seib, 1992) have been shown to inhibit retrogradation. In high-amylose starches, the amylose fraction has been suggested to have synergetic effects on the amylopectin retrogradation (Russell, 1987).

The botanical source of the starch will thus have an impact on the physico-chemical properties of starch. This influence might be attributed to the granule size distribution, to the crystallinity (degree and/or polymorphic form), to the organisation of the molecules within the granule or to the chemical nature of the starch polymers. The present investigation was undertaken in order to study in more detail the relation between physico-chemical properties of starch and the chemical nature of its constituents. Therefore, 10 different starches, representing all the polymorhpic forms (A, B and C), were chosen to cover a broad range of chemical and thermal characteristics. The starch granule size distribution was measured and, in the chemical analysis, emphasis was given to the amylose content and the amylopectin characteristics. The physico-chemical properties studied were gelatinization and retrogradation measured using differential scanning calorimetry (DSC). The gelatinization was studied at limited water content, and the retrogradation was studied under conditions to ensure extensive retrogradation (storage at 6°C). The results were evaluated by principal component analysis (PCA).

MATERIALS AND METHODS

Six cereal starches, three potato starches and one pea starch were used in this study, as outlined in Table 1. The pea and the cereal samples, except for the waxy maize, were obtained from Svalöf-Weibull AB (Landskrona, Sweden), and the starch was isolated according to Meredith et al. (1978). The three barleys were pearled prior to starch isolation in a Strong-Scott Seedburo Mill. The waxy maize starch and the three potato starches were supplied by Lyckeby-Stärkelsen (Kristianstad, Sweden).

Chemical characterization

The starch content was determined enzymatically according to Åman et al. (1994), and the dry matter by oven drying at 105°C for 16 h. The size distribution of the starch granules was determined using a Coulter Counter with a 100-channel analyser (Morrison and Scott, 1986). The channel diameters ranged from 1.98 to 60.74 μm and were converted to a linear size scale. Smoothing was applied on the distribution profiles using cubic spline interpolation, and the volumes calculated assuming spherical granules. The X-ray diffraction patterns of the pea and high-amylopectin potato starches were determined according to Svensson and Eliasson (1995). The total and apparent amylose contents were analysed by iodine staining (Morrison and Laignelet, 1983). The lipid-complexed amylose (LAM) content was calculated as the difference between the total and the apparent amylose (FAM) contents. For the three barley starches, the amylose content was calculated by the equation suggested by Tester and Morrison (1992). The amylose content was also determined by gel permeation chromatography (GPC). The samples (1.5 mg ml^{-1}) were prepared essentially as described by Torneport et al. (1990). For debranching, however, 5 μl isoamylase from *Pseudomonas amyloderamosa* (EC 3.2.1.68, 71 000 U mg^{-1} protein, obtained from Hyashibara, Biochemical Labs, Inc., Okayama, Japan) was used, and the enzyme was inactivated in a boiling water-bath for 5 min. The samples were injected on a Sepharose CL 6B column (1.6 × 70 cm) using 0.25 M KOH as eluent

at a flow rate of 13 ml h^{-1}. Two-ml fractions were collected, and the elution profile was detected by the phenol–sulphuric acid method of Dubois et al. (1956). The amylose, consisting of the long-chain α-1,4-glucans after debranching of the starch, comprised the first fraction eluted, including the void peak and the intermediate material between the amylose and the amylopectin unit-chains.

For characterization of the chain length distribution of the amylopectin, the starch was dissolved in 0.1 M NaOH. The amylopectin was isolated by GPC (Lloyd et al., 1996) and freeze-dried, prior to debranching by isoamylase and further analysis by high-performance size-exclusion chromatography (HPSEC). Amylopectin unit-chain fractions with defined average DP were used for calibration of the HPSEC system (Fredriksson et al., 1997). This calibration was also used for calculation of the weight average DP ($\overline{DP}_w$). The distribution profiles of the amylopectin unit-chains were divided into four parts, defining sub-fractions (F1–F4) with decreasing chain lengths. The limits between the different sub-fractions were determined by the inflection points at DP 82.3 (F1/F2) and DP 17.8 (F3/F4) and the minimum at DP 37.4 (F2/F3), estimated from the first-derivative curve of an average chromatogram calculated from the 10 amylopectins examined. All the chemical analyses, except the determination of the starch granule size distribution, were performed in duplicates at the least.

DSC measurements

The thermal transitions of starch were examined with a Perkin-Elmer DSC 2c (starch from wheat, rye, barley, potato Desiree and pea) or with a DSC 6200 from Seiko Instruments Inc. (starch from waxy maize, high-amylopectin potato and potato Prevalent). Coated sample pans of aluminium from TA Instruments were used with an empty sample container as reference. Starch (5–10 mg) was transferred into weighed sample containers, and the appropriate amount of water was added. The sample containers were then sealed and reweighed. The samples were allowed to equilibrate for at least 2 h before d.s.c. analysis to attain an even distribution of water. The exact water content of each sample was determined by drying the punctured container in an oven at 105°C for 24 h after the DSC scan. The starch: water ratio for all samples studied was approximately 1:1 (0.49–0.53). In the starch gelatinization studies, each sample was examined by DSC after the equilibration period. In the starch retrogradation studies, the sample containers were heated in an oven for 15 min at 105°C and then stored at 6°C for 2 or 4 days before DSC analysis. All the samples were analysed at 17–127°C, with a heating rate of 10°C min^{-1}.

Each DSC endotherm of gelatinization was characterized by the onset temperature ($T_{o,gel}$), the temperature at peak minimum ($T_{m,gel}$), the offset temperature ($T_{f,gel}$), the melting interval ($\Delta T_{gel} = T_{f,gel} - T_{o,gel}$) and the melting enthalpy (ΔH_{gel}). The DSC endotherms related to starch retrogradation were evaluated by determining the onset temperature of melting of recrystallized amylopectin ($T_{o,2}$ or $T_{o,4}$), the temperature at peak minimum ($T_{m,2}$ or $T_{m,4}$), the offset temperature ($T_{f,2}$ or $T_{f,4}$), the interval of melting ($\Delta T_2 = T_{f,2} - T_{o,2}$ and $\Delta T_4 = T_{f,4} - T_{o,4}$) and the melting enthalpy (ΔH_2 or ΔH_4). The dissociation of the amylose–lipid complex was assessed by determining the peak minimum temperature of melting of the complex (T_{cx}) and the melting enthalpy (ΔH_{cx}) of the complex. Except for ΔH_{cx}, the enthalpy values were calculated on an amylopectin basis, using the amylopectin contents obtained by the GPC procedure, and all enthalpies were expressed on a dry matter basis. All DSC results are the means of three measurements. The measured enthalpies and transition temperatures were generally within 5% of the given values.

Statistical analysis

The variations observed in the chemical and physical properties of the 10 different starches were examined by principal component analysis (PCA) with the computer software SIRIUS (Pattern Rec System A/S, N-5015, Bergen, Norway). To determine the significance of the principal components (PC), the procedure of cross-validation was used (Wold, 1978).

RESULTS AND DISCUSSION

Origin of samples and starch granule distribution

Ten starches of different botanical origin were chosen to cover a broad variation of chemical and thermal characteristics. The studied material included commonly grown starch-rich crops, such as wheat, rye, barley, waxy maize, pea and potato, as well as different genotypes from conventional breeding programs (high-amylose and waxy barley) and genetically modified starch (high-amylopectin potato).

The starch content (glucose residues) was high in all samples and ranged from 92.9 to 96.6% of dry matter. Wheat, barley and rye starches have a bimodal granule distribution (Karlsson et al., 1983; Lineback, 1984), with larger lens-shaped A-granules and smaller spherical B-granules (Fig. 1*a*). The starch isolation was carried out with the aim of mimicking commercial conditions, which resulted in some losses of material. Small starch granules tend to associate with the protein fraction and may be lost during the isolation procedure (McDonald and Stark, 1988), and the results also indicated such losses for the cereal starches, especially for the three barley starches (Oscarsson et al., 1997). The rye starch differed from the wheat starch through the broader distribution profile and the larger diameter of the A-granules. The starch granules of normal-amylose and waxy barley had similar distribution profiles, both with only traces of the B-granule fraction, and with slightly smaller A-granules than the wheat starch. The high-amylose barley had smaller A-granules than the other two barleys which was in agreement with the findings of Oscarsson et al.

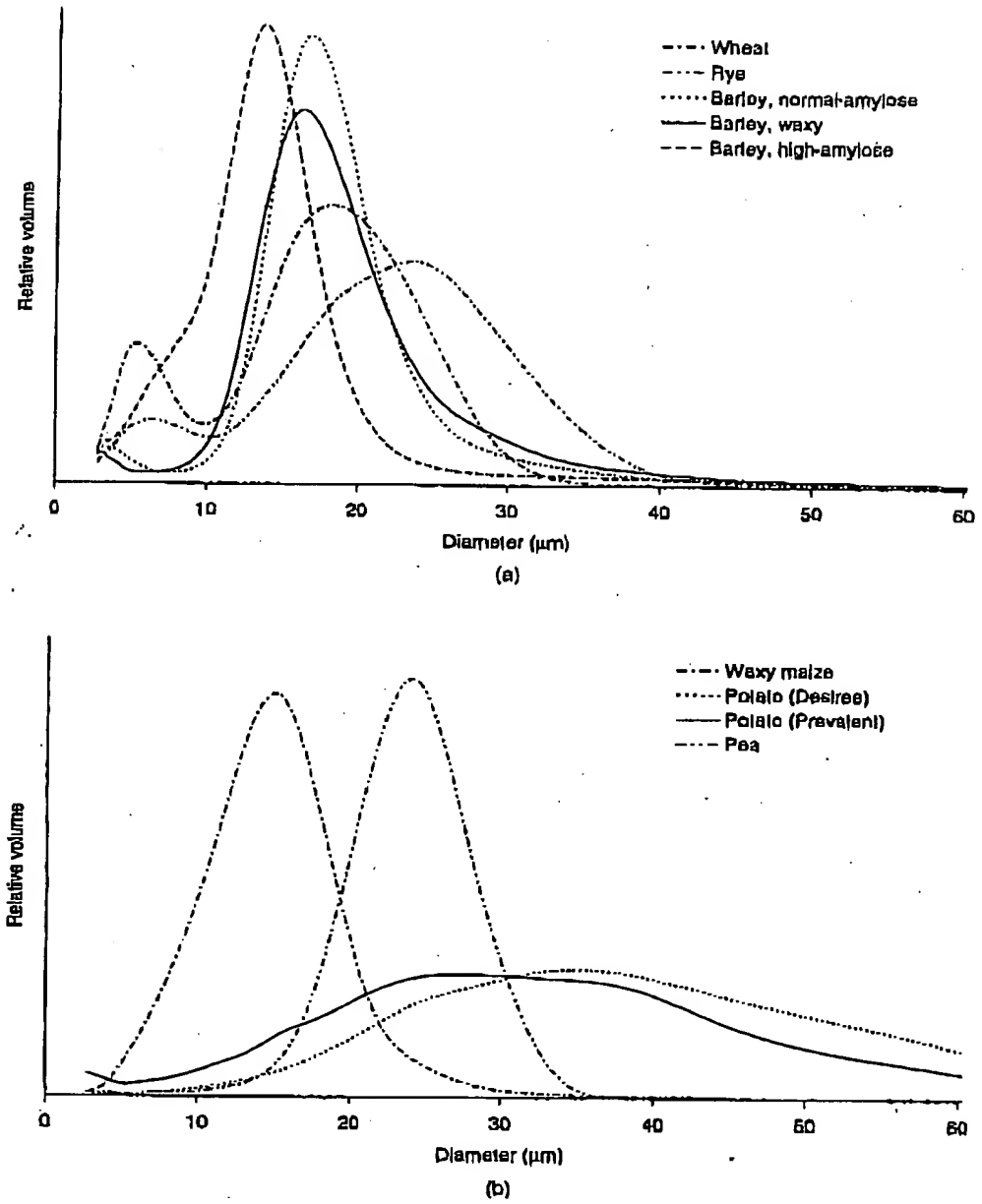

Fig. 1. Granule size distribution of starches from (*a*) wheat, rye and barley, (*b*) waxy maize, potato and pea

(1997). Waxy maize, potato (Lineback, 1984; Snyder, 1984) and pea starches (Eliasson, 1988) have monomodal starch granule distributions (Fig. 1*b*). In waxy maize, the peak maximum of the starch granule distribution profile appeared between those shown by normal- and high-amylose barley. The size of the pea starch granules was similar to that of the A-granules in rye starch, but the distribution profile was narrower. The starches from potato cultivars Desiree and Prevalent had the broadest starch granule distribution and the largest granules of the starches studied. The starch granule distribution of the high-amylopectin potato starch could not be analysed, since the instrument was blocked by aggregating or possibly cold swelling granules.

Amylose content

When determined by iodine staining, the total amylose content was at a similar level (25.0–27.4%) in the wheat, rye and normal-amylose barley starches (Table 2). The LAM content was close to 5% in both the wheat and normal-amylose barley starches but lower in the rye starch. There was a linear relationship between the total amylose and

Table 2. Amylose contents of the starch samples, as determined by iodine staining (% of dry matter) and GPC (% of sugar residues), as well as gelatinization enthalpies (J g^{-1} dry starch) and temperatures (°C) of the amyloselipid complex

	[FAM[a]]	[LAM[b]]	[Total]	[Amylose content by GPC]	ΔH_{ex}	T_{ex}
Wheat	20.4	4.6	25.0	28.4	1.4	110.1
Rye	22.7	3.3	26.0	28.6	0.8	107.8
Barley						
Normal-amylose	21.9	5.5	27.4	29.3	1.8	110.3
Waxy	3.2	2.4	5.6	7.1	0.8	112.8
High-amylose	29.6	7.5	37.1	30.0	2.8	110.8
Waxy maize	0.3	0.5	0.8	1.6	ND[c]	ND[c]
Potato						
Normal-amylose (Desiree)	28.2	-0.9	27.3	23.0	ND[c]	ND[c]
Normal-amylose (Prevalent)	24.7	-0.1	24.6	20.4	ND[c]	ND[c]
High-amylopectin	3.8	0.4	4.2	1.0	ND[c]	ND[c]
Pea	33.9	-0.5	33.4	33.7	ND[c]	ND[c]

[a]Apparent amylose
[b]Lipid-complexed amylose
[c]Not detected

LAM contents for the three barleys. One interesting characteristic of the waxy barley seems to be a high proportion of LAM in relation to the total amylose (Morrison, 1995). The amylose content of starch from potato Prevalent (24.6%) was significantly lower than that of starch from potato Desiree (27.3%). The negative LAM values obtained for Prevalent, Desiree and for pea were probably due to the uncertainty of the method. This may become more pronounced in samples with little or no native starch lipids. The amylose contents reported in this study were similar to those obtained previously using the same methodology (Morrison and Laignelet, 1983; Morrison et al., 1984; Doublier, 1987; Radosta et al., 1991).

The total amylose contents determined by GPC were comparable with those obtained by the iodine staining (Table 2). The main differences may be explained by variations in the amylopectin or amylose structure. The amylose contents obtained by GPC for the potato starches were probably more accurate than those obtained by iodine staining. In general, potato starch has longer amylopectin unit-chains than, e.g. cereal and pea starches (Kalichevsky et al., 1990). Therefore, the potato amylopectin may have contributed to the iodine staining to a larger extent than the other starches studied, resulting in somewhat overestimated amylose contents.

In high-amylose rice and maize, atypical starch materials have been detected (Asaoka et al., 1986; Inouchi et al., 1987). Therefore, the possibility of finding anomalous types of starch has been discussed also for high-amylose barley. In this study, the GPC analyses of normal- and high-amylose barley resulted in almost identical cumulative curves, when including the void peak and the intermediate material preceding the amylopectin unit-chains in the amylose fraction. This result showed that the intermediate material constituted the same proportion of the amylose in both samples. This observation is also in agreement with previous findings reported by Tester et al. (1991).

Characterization of amylopectin

The distribution of the amylopectin unit-chains generally appears to be genetically controlled and characteristic of a species (Hizukuri, 1985; Kalichevsky et al., 1990). However, some genotypes with a somewhat different amylopectin structure do exist, for example, high-amylose genotypes of maize and rice (Hizukuri, 1985; Asaoka et al., 1986; Inouchi et al., 1987). Smaller variations observed in wheat and barley amylopectin have been assigned to the growing temperature (Tester et al., 1991; Shi et al., 1994). The amylopectin chain distribution profiles for the 10 starches were examined by HPSEC, after debranching with isoamylase. All the cereal starches showed a polymodal distribution, with local peak maxima or shoulders at DP 11–12, 18–19 and 46–48 (Fig. 2). The amylopectins from potato Desiree and Prevalent also exhibited a polymodal distribution profile with peak maxima or shoulders at DP 13, 17, 50 and 79–80, whereas the profile for the high-amylopectin potato was slightly different, with peak maxima at 15, 18, 52 and 82. The amylopectin profile for pea was similar to that of the waxy maize with DP maxima of 14 and 47–49 and a shoulder at DP 19–20. Of the cereal amylopectins studied, that of the high-amylose barley had the lowest $\overline{DP}_w$ value (24.0) (Table 3), whereas the waxy maize amylopectin had the highest (28.0). The $\overline{DP}_w$ value for the pea amylopectin was 26.6. The overall highest values were obtained for the three potato amylopectins (31.4–34.8). These $\overline{DP}_w$ values were in the same range as those found in previous studies. Hizukuri (1985) investigated the relationship between the chain profiles of amylopectin and the crystalline structure of starch granules for 20 samples, including wheat, waxy maize and potato. The A-type amylopectins had $\overline{DP}_w$ of 26 (23–29), the B-type amylopectins 36 (30–44) and the C-type amylopectins around 28. Similar results were reported for barley (MacGregor and Fincher, 1993), wheat and potato (Hizukuri, 1986). Some smaller deviations between results from different studies may, at

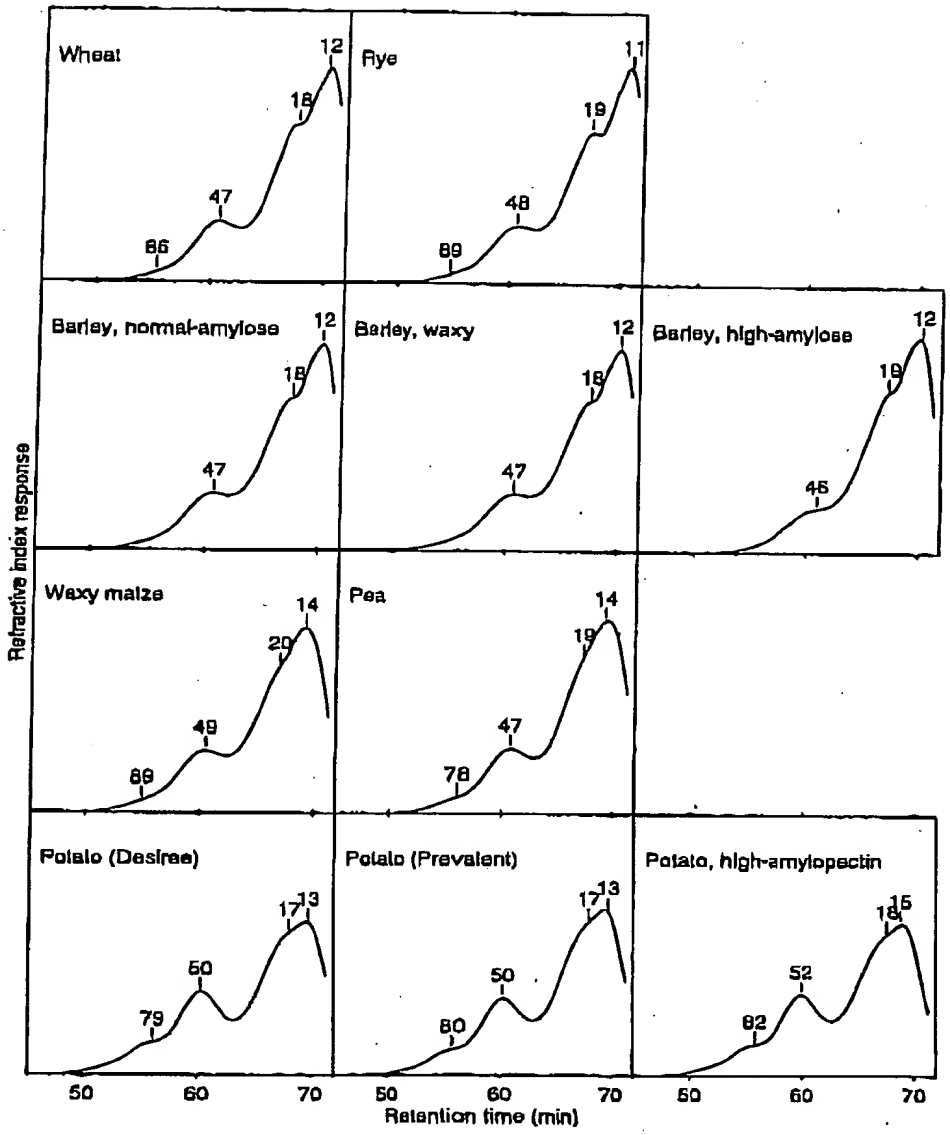

Fig. 2. Amylopectin unit-chain length distribution after debranching of the 10 starches. Arrows indicate DP at local peak maximum or shoulder

least in part, be of experimental character due to differences in the methods used but may also be of genetic or environmental origin.

The amylopectin chain length distribution profiles of the wheat, rye, normal-amylose barley and waxy barley were all very similar, with a relative chain length distribution between 20.1 and 21.3% for fractions F1 + F2 and between 78.7 and 79.8% for fractions F3 + F4, respectively (Table 3). The amylopectin from high-amylose barley contained a considerably lower proportion of fractions F1 + F2 than any of the other samples analysed. This is in line with the results by Tester et al. (1991), that also indicated smaller proportions of long amylopectin chains in high-amylose barley than in normal and waxy barley. Salomonsson and Sundberg (1994), on the other hand, obtained a higher proportion of certain longer amylopectin unit-chains (DP 30–49) in the high-amylose barley than in the normal-amylose and waxy barley. These results imply that the amylopectin fraction of high-amylose barleys to some extent may differ in structure. The three potato amylopectins had the largest proportions of fractions F1 + F2 containing long amylopectin unit-chains, but the proportion was slightly smaller in potato Prevalent than in potato Desiree and the high-amylopectin potato. However, this result indicates that the amylopectin structure is similar in all three potato starches. The amylopectin distribution profiles of the waxy maize and pea were intermediate between those of the aforementioned starches. Hizukuri (1986) observed a

Table 3. Amylopectin content of the isolated starch samples, as determined by GPC (% of dry matter), relative distribution of amylopectin unit-chains (%) and weight-average degree of polymerization ($\overline{DP}_w$) of debranched amylopectins

	[Amylopectin content]	[Chain length distribution]						$\overline{DP}_w$
		F1	F2	F3	F4	[F1 + F2]	[F3 + F4]	
Wheat	71.6	1.3	18.8	34.1	45.7	20.1	79.8	25.7
Rye	71.4	1.7	18.7	34.6	45.0	20.4	79.6	26.2
Barley								
Normal-amylose	70.7	1.2	19.2	34.8	44.8	20.4	79.6	25.8
Waxy	92.9	1.9	19.4	34.6	44.1	21.3	78.7	26.7
High-amylose	61.0	0.6	15.5	36.7	47.3	16.1	84.0	24.0
Waxy maize	98.4	2.1	21.6	35.6	40.8	23.7	76.4	28.0
Potato								
Normal-amylose (Desiree)	77.0	6.1	28.1	30.6	35.3	34.2	65.9	33.9
Normal-amylose (Prevalent)	79.6	4.4	25.6	32.7	37.4	30.0	70.1	31.4
High-amylopectin	99.0	6.5	28.1	33.1	32.4	34.6	65.5	34.8
Pea	66.3	1.6	20.7	33.9	43.8	22.3	77.7	26.6

polymodal amylopectin distribution profile having five peaks; A, B1, B2, B3 and B4 and suggested that these populations may be involved in the formation of up to four different clusters. In the present study, fraction F1 approximately corresponds to peaks B3 and B4, F2 to B2, F3 to B1 and F4 to A. However, since this type of analysis is based on decomposition of the amylopectin molecule it is hard to reconstruct the actual structure. Even though the distribution profiles were more or less identical for several of the starches studied, the appearance of the intact amylopectin molecules may still differ.

Starch gelatinization

The gelatinization of the various starches was studied by DSC at a water content of approximately 50%. Due to the limited water content, the typical broadening on the high-temperature side of the gelatinization endotherm was evident for all the samples studied (Eliasson, 1980) (Fig. 3). However, the position and appearance of this broadening varied and, consequently, affected the temperature range of gelatinization. The separation between this high-temperature shoulder and the first part of the endotherm was most apparent for the pea starch, whereas for the three potato starches, the separation was poor.

The gelatinization enthalpy (ΔH_{gel}) is a measure of the overall crystallinity of the amylopectin, i.e. the quality and quantity of starch crystals (Tester and Morrison, 1990b). Pure crystalline A-spherulites exhibit a melting enthalpy of approximately 35 J g^{-1}, which is very similar to the melting enthalpy for pure crystalline B-spherulites (Whittam et al., 1990). For native starches, the molecular order in form of double helices is significantly higher than the crystalline order (Gidley, 1985). In cereal starches and tapioca, but not in potato starch, both levels of structure, i.e. double-helical and crystalline, are disrupted concurrently during gelatinization, and it has been suggested that ΔH_{gel} primarily reflects the loss of double-helical order (Cooke and Gidley, 1992). The amount of double-helical order in the native starches should be strongly correlated to the amylopectin content, and the crystallinity in the granules increases with the amylopectin content (Gernat et al., 1993). Therefore, all ΔH_{gel} values in this study were calculated on an amylopectin basis. These values of ΔH_{gel} were between 15.6 and 23.9 J g^{-1} amylopectin (Table 4). The highest values were obtained for potato cultivars Prevalent and Desiree, the lowest for the cereals, while pea and high-amylopectin potato showed intermediate values. Although the enthalpy values for pure A- and B-spherulites are nearly the same, it seemed that, even when calculated on an amylopectin basis, the ΔH_{gel} values for the cereal starches were considerably lower than those for the potato and pea starches.

The minor differences between wheat and potato starches in the data of Cooke and Gidley (1992), representing double-helical order, appear to be insufficient to explain the large differences between the ΔH_{gel} values found in the present study.

During the gelatinization, part of the free lipids present in the cereal starches will probably form a helical inclusion complex with the amylose molecules. This complex formation is an exothermic process and will result in a decrease in the observed endothermic gelatinization enthalpy (Eliasson, 1986). This may be one reason why the ΔH_{gel} values for the pea and potato starches (which do not contain any native lipids) are higher than those for the lipid-containing cereal starches. However, it does not explain why the ΔH_{gel} values were higher for wheat than for normal-amylose barley, despite the similar contents of LAM and FAM in these two cereals.

In starch, a biopolymer of partially crystalline nature, the melting of the crystallites cannot start before the glass transition of the glassy regions is exceeded. The second-order glass to rubber transition is accompanied by an incremental change in heat capacity (C_p), preceding the gelatinization endotherm during a d.s.c. scan. For rice starch, Biliaderis et al. (1986) estimated C_p for this glass transition to be 0.11 ± 0.01 J/gK. During the estimation of the ΔH_{gel} values for the 10 starches in the present study, no consideration has been taken to any possible glass transition related to the melting endotherms, i.e. ΔH_{gel} was calculated

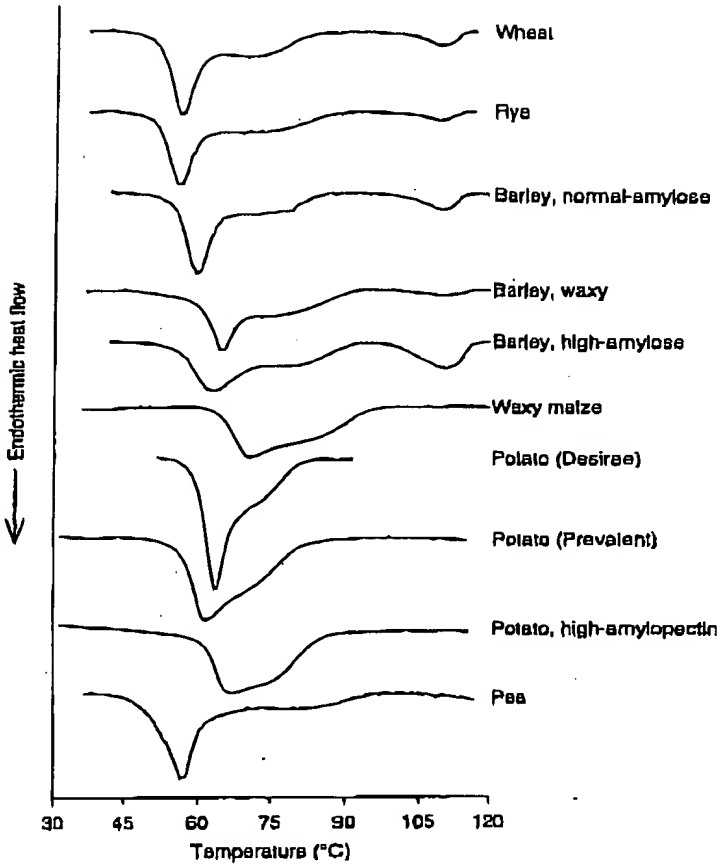

Fig. 3. DSC gelatinization endotherms of the 10 starches studied at a starch:water ratio of approximately 1:1

by estimation of the area under the endotherm with a fitted straight base line.

The gelatinization always takes place over a temperature interval (ΔT_{gel}), which in excess water may be 1–2°C for a single granule, and for the whole population of granules the interval may be >10°C (Eliasson and Gudmundsson, 1996). At intermediate or high water content, the onset (T_o) and peak minimum temperatures (T_m) are independent of the water content, whereas the offset temperature (T_f) is much influenced by the water available during gelatinization (Eliasson, 1980). In the present investigation, the highest $T_{o,gel}$ values, 59.6–64.4°C, were obtained for the high-amylopectin varieties of both A- and B-type starch (Fig. 4). This was consistent with the hypothesis that crystallinity increases with amylopectin content (Gernat et al., 1993). Starch from potato Desiree also showed a relatively high $T_{o,gel}$ value. Starches from potato Prevalent, normal-amylose barley and high-amylose barley exhibited an intermediate $T_{o,gel}$, while the lowest $T_{o,gel}$ was recorded for the pea, rye, and wheat starches. Shi and Seib (1992) investigated starches of the A-type from four waxy cereals, viz. maize, barley and two different rice samples, and found that the gelatinization temperatures (onset, peak and offset) in 75 wt.% water increased in the same order as their X-ray crystallinity. In their study, $T_{o,gel}$ for the maize and barley was 67.0 and 58.0°C, respectively, which roughly seems to be in line with the results in the present study. They concluded that the molecular structure of the microcrystalline region is the same in the four granular starches, and that the melting temperature of the crystallites in these native waxy starches is controlled indirectly by the surrounding amorphous regions. By contrast, an investigation of maize

Table 4. Enthalpy of gelatinization (ΔH_{gel}) and retrogradation related enthalpies of the starch samples after storage for 2 (ΔH_2) and 4 days (ΔH_4) at 6°C (J g^{-1} amylopectin)

	ΔH_{gel}	ΔH_2	ΔH_4
Wheat	17.5	8.1	9.8
Rye	15.8	7.3	8.7
Barley			
Normal-amylose	15.6	10.3	10.6
Waxy	15.7	10.6	11.1
High-amylose	16.1	12.5	13.1
Waxy maize	17.6	12.3	13.0
Potato			
Normal-amylose (Desiree)	22.1	13.3	12.9
Normal-amylose (Prevalent)	23.9	13.6	14.4
High-amylopectin	19.7	13.6	13.5
Pea	20.8	12.5	12.5

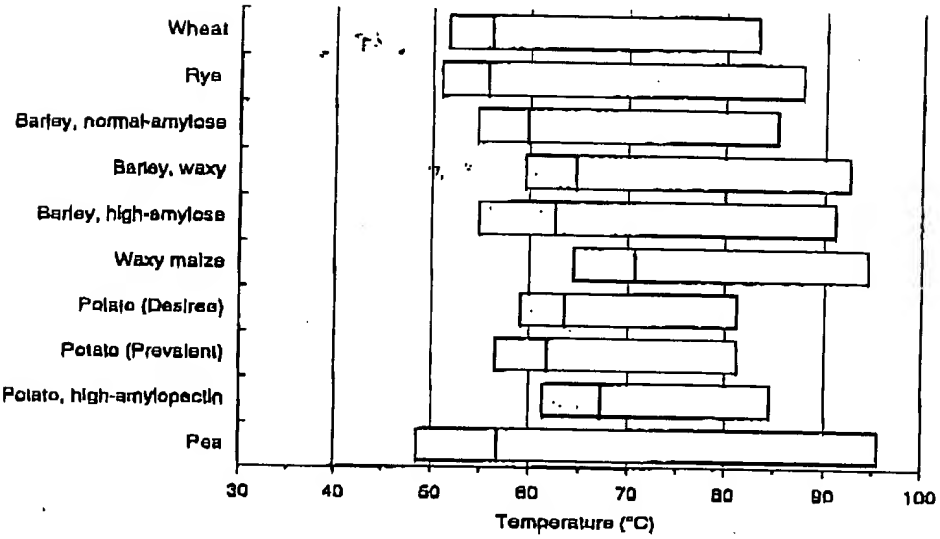

Fig. 4. Bars indicating the temperature range of gelatinization (T_o to T_f) of starch samples, border verifies the position of T_m

starches with varying amylose content (0–66.1%) indicated that gelatinization temperatures increased with increasing amounts of amylose (Knutson, 1990). A study of 11 different waxy rice starches with varying gelatinization properties showed that starches with low gelatinization temperatures had more amorphous and less crystalline material compared to starches with high gelatinization temperatures (Tester and Morrison, 1990b). Their explanation to this phenomenon was that crystallite perfection could be the principal mechanism controlling the gelatinization temperatures. Other factors, such as the amount of damaged starch, isolation techniques (Morrison, 1995) and variation in climatic conditions during growth (Shi et al., 1994; Tester et al., 1991), also influence these temperatures.

The pea starch exhibited the largest ΔT_{gel} (46.9°C) and the three potato starches the smallest (22.1–24.6°C). No correlations were found between the granule size distribution and d.s.c. parameters, such as $T_{o,gel}$, $T_{m,gel}$, ΔT_{gel} and ΔH_{gel}.

Starch retrogradation

The features of the retrogradation endotherms were very similar after 2 and 4 days of storage at 6°C, except that the areas of the DSC peaks were somewhat smaller after the shorter storage time. Hence, only the endotherms after 4 days of storage are shown (Fig. 5). The endotherms for starches from wheat, rye, normal-amylose barley and high-amylose barley were bell-shaped, and that for waxy barley starch was slightly bimodal. The endotherms for the three potato starches were all bimodal as well as those for the waxy maize and pea starches. The mono- and bimodal features of the retrogradation endotherms can be related to differences in the quality of the recrystallized amylopectin crystals, a factor which may depend on the botanical origin of the starch. The bimodal shape of the aforementioned retrogradation endotherms could also be an evidence of an amylopectin reorganization that takes place during the d.s.c. scan. Because of increased molecular mobility, there will be a chance for chain rearrangements in the amylopectin crystallites just above the onset temperature (Biliaderis et al., 1986). The structure of the recrystallized amylopectin will approach a new equilibrium, and a fraction of the crystallites will melt at a slightly higher temperature.

The melting enthalpy (ΔH_2 and ΔH_4) of recrystallized amylopectin was calculated from the area of the retrogradation-related endotherm and is expressed on an amylopectin basis (Table 4). After storage for 2 days at 6°C the starches of rye and wheat exhibited the lowest ΔH_2 values (7.3 and 8.1 J g^{-1} amylopectin, respectively). The highest ΔH_2 values were obtained for the three potato starches (13.3–13.6 J g^{-1} amylopectin) followed by the pea and high-amylose barley starches. These results confirmed earlier findings that amylopectins from cereals retrograde to a lesser extent than pea (Kalichevsky et al., 1990) and potato amylopectins (Kalichevsky et al., 1990; Silverio et al., 1996). This has been attributed to the shorter average chain lengths in the cereal amylopectins (Orford et al., 1987; Kalichevsky et al., 1990). In contrast to their results, the ΔH_2 and ΔH_4 values obtained for the pea starch were lower than those for the three potato starches.

After 4 days of storage at 6°C, a similar ranking of the different samples as that obtained after storage for 2 days was observed (Table 4) but it seems that the rate of retrogradation was slower for the wheat and the rye starches compared to the others. The varying retrogradation enthalpies, determined in the present study, reflected the large differences between the overall quality and quantity of the recrystallized amylopectin in starches of different botanical origin. Wide-angle X-ray diffraction studies (WAXS) of

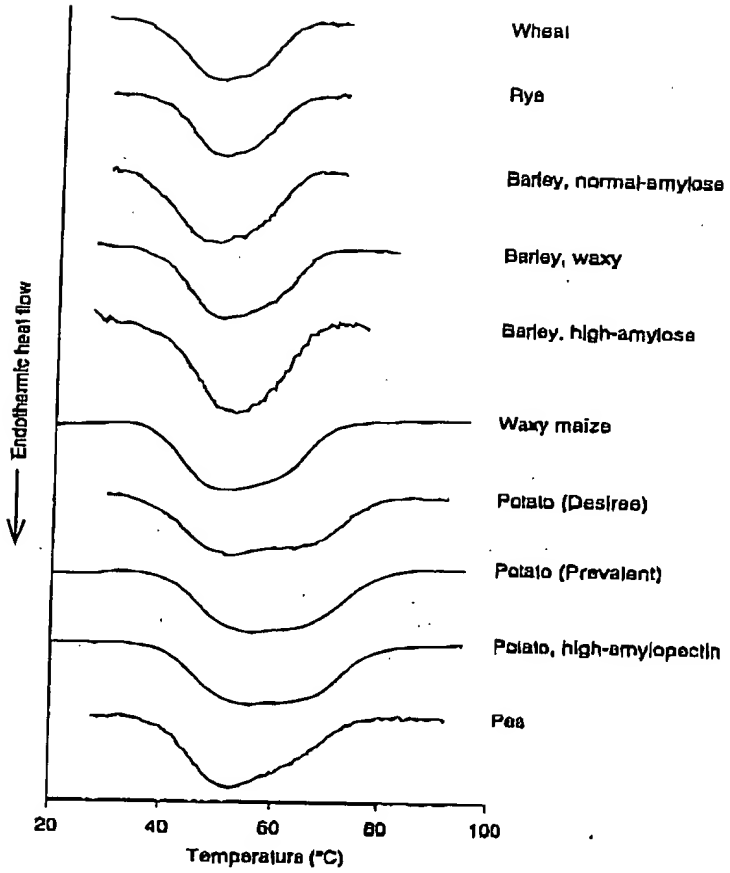

Fig. 5. DSC endotherms of recrystallized amylopectin from starch samples, after gelatinization at a starch:water ratio of approximately 1:1 and storage for 4 days at 6°C

cooked starch showed a slow development of the B form of crystallinity (Katz, 1934), which is independent of the polymorphic form the native starch had prior to gelatinization. Although retrograded amylopectin from different sources evidently shows the same B-polymorphic crystal pattern, other factors, such as structural differences in the amylopectin molecule, could cause the variations found in the rate and extent of amylopectin recrystallization. It is clear that high ΔH_{gel} values resulted in high ΔH_2 and ΔH_4 values, as obtained for the potato starches, while low ΔH_{gel} values gave low ΔH_2 and ΔH_4 values, as obtained for starches from cereals, such as rye and wheat.

It could be expected that the three barley starches should retrograde to the same extent, especially since the enthalpy of retrogradation was expressed on an amylopectin basis. The present investigation showed that the normal-amylose and the waxy barley starches indeed retrograded to the same level, but that the high-amylose barley starch retrograded to a higher extent. This observation may increase the interest for high-amylose barley from a nutritional point of view. Retrograded waxy maize starch has been shown to be more resistant to α-amylase than freshly gelatinized starch (Eerlingen et al., 1994), and may partly escape digestion and absorption in the human small intestine. In a retrogradation study of gels from nongranular mixtures of amylose and amylopectin in different ratios, synergistic interactions were seen between amylopectin and amylose at high amylose contents, i.e. unexpectedly high melting enthalpies were obtained for gels with very high amylose content (75–90%) (Gudmundsson and Eliasson, 1990). The possibility of cocrystallization of the two polymers has been proposed in relation to retrogradation, when amylose is found in high amounts (Russell, 1987).

Crystallization, the rate-limiting step in retrogradation, is a nucleation-controlled growth process, and the advantage of low-temperature storage is that the nucleation rate increases exponentially with decreasing temperature down to the glass transition. On the other hand, crystal growth is favoured at a temperature just below T_m, and the rate of propagation also increases exponentially with increasing temperature up to T_m (Slade and Levine, 1987). The melting temperature range (ΔT_2 and ΔT_4) gives an indication of the quality and heterogeneity of the recrystallized amylopectin. Thus, a wide melting range might imply a large amount of crystals of varying stability, whereas a narrow range could suggest crystals of a more homogeneous quality and similar

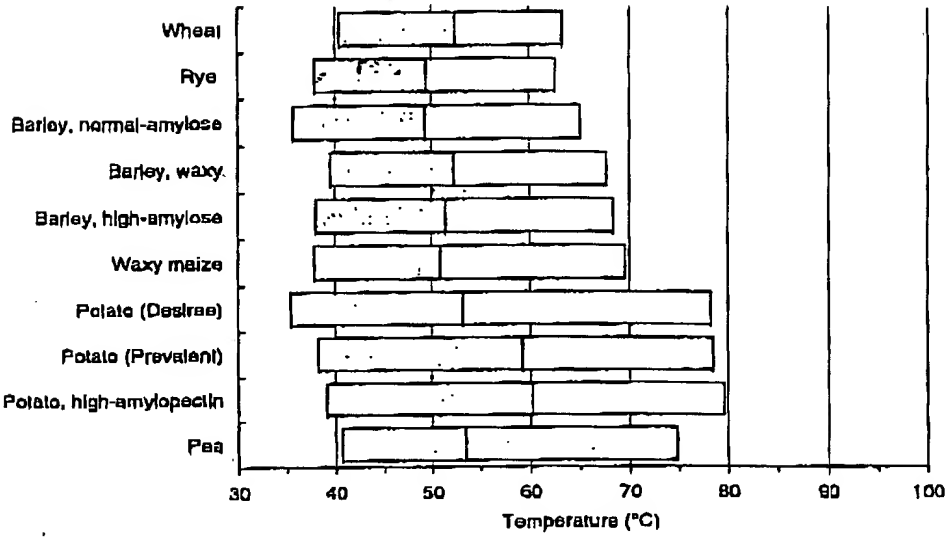

Fig. 6. Bars indicating the melting temperature ranges ($T_{o,2}$ to $T_{f,2}$) of recrystallized amylopectin after 2 days of storage at 6°C, border verifies the position of $T_{m,2}$

stability. The widest melting ranges (40.3–43.3°C) were obtained for the three potato starches (Figs. 6 and 7), indicating that a heterogeneous mixture of amylopectin crystals of differing stability was formed during ageing of these starches. Interestingly, the potato starches also exhibited the highest gelatinization and retrogradation enthalpies. By contrast, the potato starches showed the narrowest melting range (ΔT_{gel}) during gelatinization. During biosynthesis, the potato starch granules may therefore create more homogeneous crystal regions than the other starches studied. Fairly large melting ranges (ΔT_2 and ΔT_4) were also observed for the pea and waxy maize starches. For the pea starch, a wide melting range (ΔT_{gel}) was also observed during gelatinization. For the remaining starches, ΔT_2 was 22.8–30.4°C and ΔT_4 25.7–29.9°C.

The onset temperature, $\Delta T_{o,2}$ ranged from 35.4 to 40.7°C and $\Delta T_{o,4}$ from 35.1 to 39.6°C. The temperatures $\Delta T_{m,2}$ and $\Delta T_{m,4}$ at peak minimum were 49.4–60.3 and 47.8–60.1°C, respectively. The variation within the $T_{o,2}$ and $T_{o,4}$ values (*ca.* 5°C) for the various retrograded starches was fairly small compared to the differences among the $T_{o,gel}$ values (*ca.* 16°C) for the native starches. This is probably due to the mechanism by which the storage temperature controls the melting temperature of the least stable recrystallized amylopectin crystal. At a particular storage temperature, only a certain amount of crystals of similar stability is formed. The

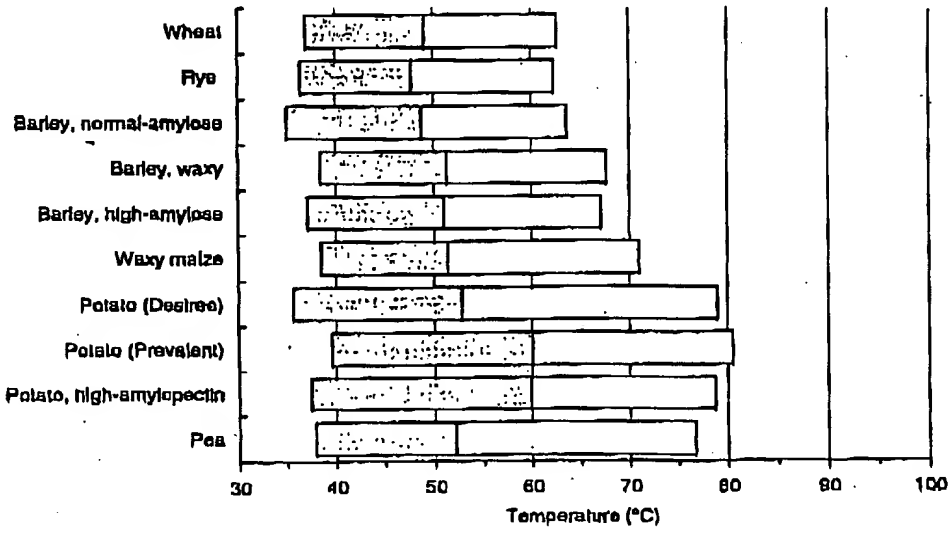

Fig. 7. Bars indicating the melting temperature ranges ($T_{o,4}$ to $T_{f,4}$) of recrystallized amylopectin after 4 days of storage at 6°C, border verifies the position of $T_{m,4}$

$\Delta T_{m,4}$ were all lower than ΔT_{gel} for each of the 10 starches studied and varied between 1.7°C for potato Prevalent and 19.4°C for the waxy maize. This is all in accordance with the theory of predicting the behaviour of recrystallization and melting of fully water-plasticized starch gels (Slade and Levine, 1987).

Amylose–lipid complex

A transition endotherm due to the melting of the amylose–lipid complex was observed for the wheat, rye and three barley starches (Table 2). The highest transition enthalpy (ΔH_{ex}) was obtained for the high-amylose barley starch, while starches from the waxy barley and rye showed the lowest enthalpies. The melting endotherms of these cereal starches were related to the LAM content. It has been suggested that the retarding mechanism of lipids on starch retrogradation is connected to their ability of forming an inclusion complex with the amylose fraction (Krog and Nybo-Jensen, 1970). The striking results obtained in the present investigation, regarding the amylose–lipid complex transitions, were that the rye starch, showing the lowest ability to retrograde, had the lowest ΔH_{ex} value, while the high-amylose barley starch, having the highest LAM content, exhibited a high retrogradation capacity. Despite the traces of LAM detected in the starches from the waxy maize and the high-amylopectin potato, no melting endotherm was detected for these starches. This was also the case for the pea starch and the two remaining potato starches. The traces of LAM possibly present in these starches may partly explain their high retrogradation capability.

The peak minimum temperature (T_{ex}) for the melting transition ranged from 108.0 to 112.3°C.

Principal component analysis—overview of the material

PCA was used to visualise the variation in amylose and amylopectin characteristics as well as in gelatinization and retrogradation properties (Table 5). With this statistical method, a large number of variables are reduced to a few orthogonal variables called principal components (PC), which describe the greatest covariance in the data analysed. The first and the second PC, describing 56 and 18% of the variance, respectively, provided an overview of the starch samples. The three potato starches had high positive scores in PC1, whereas the cereal starches, except that from the waxy maize, had negative scores (Fig. 8*a*). The starches from rye, wheat, normal-amylose barley and high-amylose barley were found close to PC1, indicating little influence from PC2. The three high-amylopectin starches, had negative scores in PC2. The pea starch had a high positive score in PC2. The starches from pea, waxy barley and waxy maize were located close to zero in PC1.

The loading plot of the two first PC described 74% of the variance in the chemical and thermo-analytical variables (Fig. 8*b*). The variance explained by these two PC was above 60% for all variables, except for T_{gel}, $T_{f,gel}$, $T_{o,2}$ and $T_{o,4}$, indicating no correlation between the latter four variables and any other studied variable. Variables found close to each other in pairs or groups indicate a positive correlation. The group of variables describing $\overline{DP}_w$, the size of the two amylopectin fractions consisting of long unit-chains (F1 and F2), ΔH_2, ΔH_4, ΔT_2, ΔT_4, $T_{m,2}$, $T_{m,4}$, $T_{f,2}$ and $T_{f,4}$, as well as ΔH_{gel}, confirmed previous findings that a high $\overline{DP}_w$ favours retrogradation (Orford et al., 1987; Kalichevsky et al., 1990). This relationship is illustrated by the correlation between the sum of the amylopectin fractions F1 and F2 and ΔH_4 in Fig. 9*a*. The loading plot also showed that $\overline{DP}_w$ correlated more weakly to ΔH_{gel} than to ΔH_2 and ΔH_4, even if the trend was the same. In addition, $T_{o,gel}$ and $T_{m,gel}$ were negatively correlated to the amylose content since these variables (AmG, AmI and FAM), were found on the opposite side of a diagonal (intersecting origo) in the plot. Fig. 9*b* shows the negative correlation between AmG and $T_{o,gel}$. Variables found in orthogonal directions, as indicated by the arrows in Fig. 8*b*, varied independently of each other. Thus the amylose content and the gelatinization temperatures ($T_{o,gel}$, $T_{m,gel}$) varied independently from variables describing the amylopectin unit-chain distribution, the LAM content and several of the gelatinization and retrogradation variables (Fig. 8*b*).

When comparing the score and loading plots (Fig. 8*a*,*b*),

Table 5. The different variables examined with PCA

Variable	Description
FAM	Lipid-free amylose
LAM	Lipid-complexed amylose
AmI	Amylose content, iodine staining
AmG	Amylose content, GPC
ΔH_{gel}	Gelatinization enthalpy
$T_{o,gel}$	Onset temperature of gelatinization
$T_{m,gel}$	Peak min. temperature of gelatinization
$T_{f,gel}$	Offset temperature of gelatinization
ΔT_{gel}	Temperature interval of gelatinization
ΔH_2	Retrogradation enthalpy, 2 days at 6°C
$T_{o,2}$	Onset temperature of retrogradation endotherm, 2 days at 6°C
$T_{m,2}$	Peak min. temperature of retrogradation endotherm, 2 days at 6°C
$T_{f,2}$	Offset temperature of retrogradation endotherm, 2 days at 6°C
ΔT_2	Temperature interval of retrogradation endotherm, 2 days at 6°C
ΔH_4	Retrogradation enthalpy, 4 days at 6°C
$T_{o,4}$	Onset temperature of retrogradation endotherm, 4 days at 6°C
$T_{m,4}$	Peak min. temperature of retrogradation endotherm, 4 days at 6°C
$T_{f,4}$	Offset temperature of retrogradation endotherm, 4 days at 6°C
ΔT_4	Temperature interval of retro gradation endotherm, 4 days at 6°C
F1	Relative amylopectin unit-chain length distribution, fraction with longest chains
F2	Relative amylopectin unit-chain length distribution
F3	Relative amylopectin unit-chain length distribution
F4	Relative amylopectin unit-chain length distribution, fraction with shortest chains
DP_w	Average DP after debranching of the amylopectin, calculated on weight basis

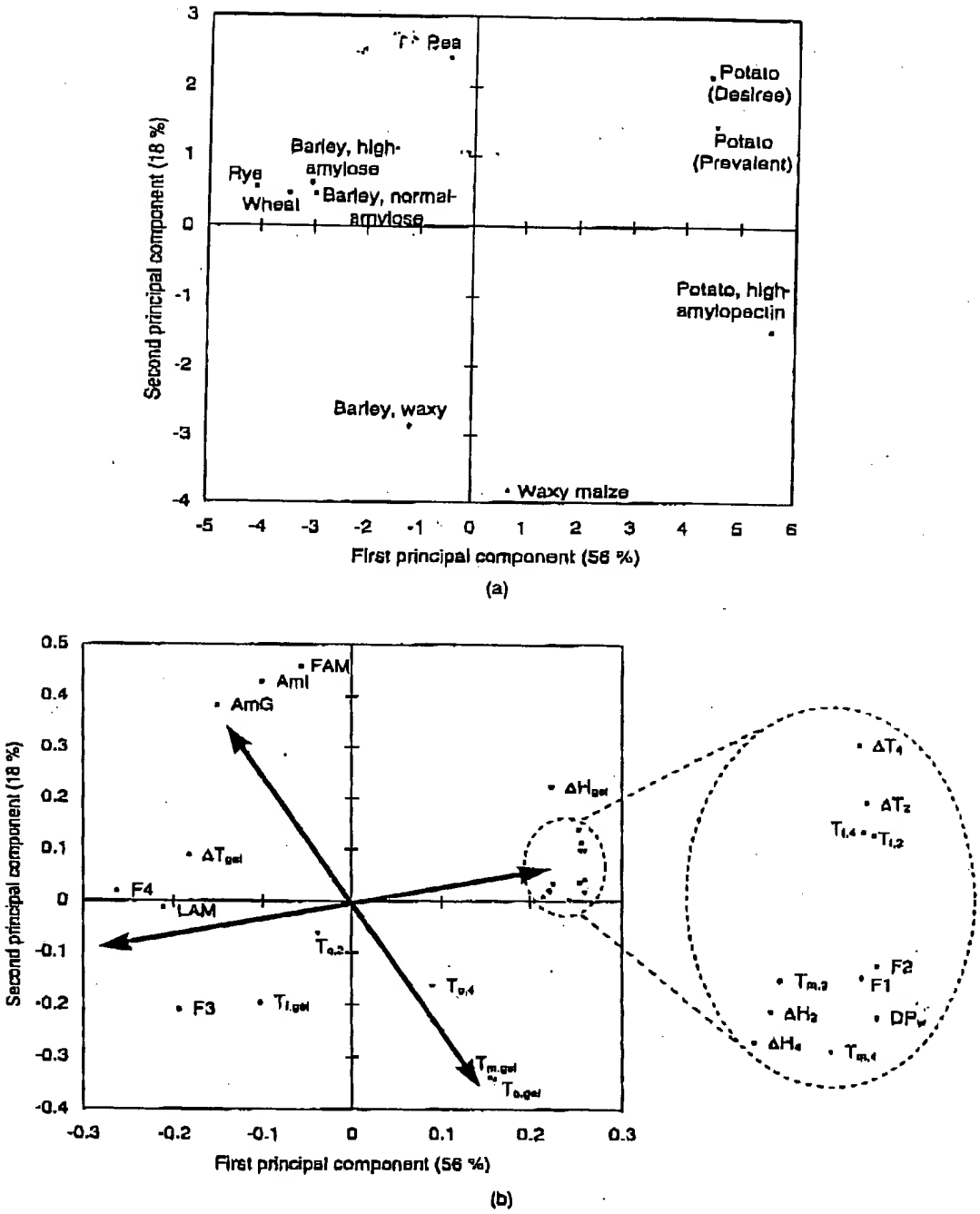

Fig. 8. (*a*) Score plot and (*b*) loading plot of the principal components 1 and 2, describing the variation in chemical and thermal properties of the 10 starch samples. Arrows indicate main trends in correlations between the different measured variables in the studied material

the three potato starches showed high $\overline{DP}_w$ values, large proportions of fractions F1 and F2, as well as high values of ΔH_2, ΔH_4, ΔT_2, ΔT_4, $T_{m,2}$, $T_{m,4}$, $T_{f,2}$ and $T_{f,4}$ and ΔH_{gel}. On the other hand, the cereal starches with a normal or high amylose content, had high negative scores in PC1 because of large fractions of F3 and F4, the presence of LAM and low values of those thermo-analytical variables that were high for the potato starches. The negative scores in PC2 for the waxy maize and waxy barley starches mainly corresponded to the absence of amylose and the high gelatinization temperatures. The pea and the waxy maize starches had scores close to zero in PC1, since the chain distribution of their amylopectin unit-chains was in between those of the potato and the other starches studied.

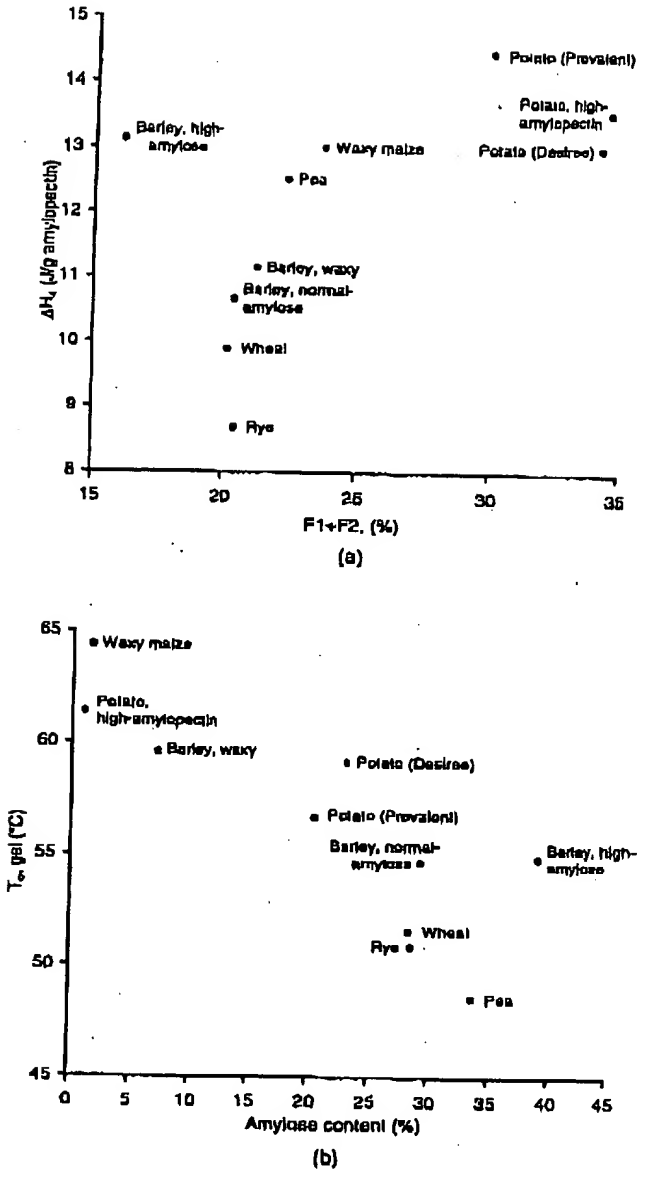

Fig. 9. (*a*) Retrogradation enthalpies (ΔH_4), after 4 days of storage at 6°C, as a function of the sum of amylopectin fractions F1 and F2. (*b*) Onset temperature of gelatinization (T_o) as a function of the amylose content (AmG)

Some of the gelatinization and retrogradation variables describe similar features but were found apart in the loading plot. The melting temperatures of native and retrograded starches both measure the quality and heterogeneity of the amylopectin crystals. For the retrograded starch, $T_{f,2}$ and $T_{f,4}$ were positively correlated to ΔT_2 and ΔT_4, respectively, since $T_{o,2}$ and $T_{o,4}$ were at a similar level in all samples. A different pattern was found for native starch, where both $T_{o,gel}$ and $T_{f,gel}$ varied among the various starches, and their correlation to ΔT_{gel} was poor. Furthermore, the retrogradation enthalpies (ΔH_2 and ΔH_4) were positively correlated to the melting intervals (ΔT_2 and ΔT_4), which was not the case during gelatinization. The main reason for these differences is probably, as already discussed in the starch retrogradation section, that the crystallization processes of biosynthesis and retrogradation are influenced by different factors. During retrogradation, the storage temperature was constant, whereas the biosynthetic pathway is far more complex and influenced by several other factors.

Although the results of the chemical analyses revealed a close resemblance in the amylopectin chain length distribution and the amylose content, the cereal starches (wheat, rye and barley) with a normal amylose content showed some individual variations, both in gelatinization and retrogradation properties. The differences in ΔH_{gel} were similar to previously published results (Kalichevsky et al., 1990; Radosta et al., 1991). One explanation of the variations in ΔH_{gel} could be the differences in LAM content, if the formation of the amylose–lipid complex causes a reduction in the endothermic heat flow (Eliasson, 1986). However, in the present study the lowest ΔH_{gel} value was obtained for the rye starch, although the LAM content of this starch was lower than that of the wheat and barley starches. The high-amylose barley had notably high retrogradation enthalpies, despite the presence of only a small proportion of long amylopectin unit-chains. With no anomalous starch material found, one possible explanation could be synergistic interactions between amylopectin and amylose at high amylose contents (Russell, 1987). Another reason could be the large proportion of the amylopectin fraction F4 (DP < 17.8). A contradictory observation was the high LAM content, which also ought to retard retrogradation. Apart from the differences in gelatinization temperatures, the high-amylopectin starches of barley and potato seemed to have thermal properties similar to those of their normal-amylose counterparts. Similar results have been reported previously for barley, maize (Morrison, 1995) and wheat (Yasui et al., 1996). In products and processes where the amylose may have detrimental effects, the high-amylopectin starches should be favoured.

CONCLUSION

The 10 starches studied provided valuable information about various starch properties and characteristics. Using PCA, it was possible to identify two main trends (indicated by arrows in Fig. 8*b*) and several correlations as well as differences in both chemical and thermal data. One of the trends observed was the correlation between the amylopectin unit-chain distribution and the thermo-analytical variables describing the retrogradation melting intervals, peak minimum and offset temperatures as well as the gelatinization and retrogradation enthalpies. The other trend was the negative correlation between the amylose content and the gelatinization onset and peak minimum temperatures of the starches. The fact that these two correlations were observed in almost orthogonal directions in the loading

plot indicated that they described two independent features of the starch samples.

The large sample diversity made evaluation rather complicated. In order to further elucidate and understand the links between the chemical and functional properties of starch, more knowledge is needed. The mode and location of branching need to be clarified, as well as the molecular weights of the amylose and amylopectin molecules in order to obtain a more complete understanding of the starch structure. In future investigations, new genotypes of existing starch-rich crops with a wide range of amylose/amylopectin ratios may provide useful materials to study.

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REFERENCES

Åman, P., Westerlund, E., Theander, O. (1994). Determination of starch using thermostable α-amylase. In J. N. BeMiller, D. J. Manners, R. J. Sturgeon (Eds.), *Methods in Carbohydrate Chemistry*, Vol. X (pp. 111–115). New York: Wiley.

Asaoka, M., Okuno, K., Sugimoto, Y., Yano, M., Omura, T., Fuwa, H. (1986) Characterization of endosperm starch from high-amylose mutants of rice (*Oryza sativa* L.) *Starch/Stärke*, 38, 114–117.

Biliaderis, C.G., Page, C.M., Maurice, T.J., Juliano, B.O. (1986) Thermal characterization of rice starches: A polymeric approach to phase transitions of granular starch *J. Agric. Food Chem.*, 34, 6–14.

Cooke, D., Gidley, M.J. (1992) Loss of crystalline and molecular order during starch gelatinization: origin of the enthalpic transition *Carbohydr. Res.*, 227, 103–112.

Doublier, J.L. (1987) A rheological comparison of wheat, maize, faba bean, and smooth pea starches *J. Cereal Sci.*, 5, 247–262.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. (1956) Colorimetric method for determination of sugars and related substances *Anal. Chem.*, 28, 350–356.

Eerlingen, R.C., Jacobs, H., Delcour, J.A. (1994) Enzyme-resistant starch. V. Effect of retrogradation of waxy maize starch on enzyme susceptibility *Cereal Chem.*, 71(4), 351–355.

Eliasson, A.-C. (1980). Effect of water content on the gelatinization of wheat starch *Starch/Stärke* 270–272.

Eliasson, A.-C. (1985). Retrogradation of starch as measured by differential scanning calorimetry. In R. D. Hill, L. Munck, (Eds.), *New Approaches to Research on Cereal Carbohydrates* (pp. 93–98). Amsterdam: Elsevier.

Eliasson, A.-C. (1986) On the effects of surface active agents on the gelatinization of starch—a calorimetric investigation *Carbohydr. Polym.*, 6, 463–476.

Eliasson, A.-C. (1988) Physical and chemical characteristics of legume starches *Animal Plant Sci.*, 1, 89–94.

Eliasson, A.-C., Gudmundsson, M. (1996). Starch: Physicochemical and functional aspects. In A.-C. Eliasson (Ed.), *Carbohydrates in Food*. (pp. 431–503). New York: Marcel Dekker Inc.

Eliasson, A.-C., Ljunger, G. (1988) Interactions between amylopectin and lipid additives during retrogradation in a model system *J. Sci. Food Agric.*, 44, 353–361.

Englyst, H.N., Kingman, S.M., Cummings, J.H. (1992) Classification and measurement of nutritionally important starch fractions *Eur. J. Clin. Nutr.*, 46, 33–50.

Fredriksson, H., Andersson, R., Koch, K., Åman, P. (1997) Calibration of a size-exclusion chromatography system by using fractions with defined amylopectin unit chains *J. Chromatogr. A*, 768, 325–328.

Gernat, C., Radosta, S., Anger, H., Damaschun, G. (1993) Crystalline parts of three different conformations detected in native and enzymatically degraded starches *Starch/Stärke*, 45, 309–314.

Gidley, M.J. (1985) Quantification of the structural features of starch polysaccharides by N.M.R. spectroscopy *Carbohydr. Res.*, 139, 85–93.

Gudmundsson, M., Eliasson, A.-C. (1990) Retrogradation of amylopectin and the effects of amylose and added surfactants/emulsifiers *Carbohydr. Polym.*, 13, 295–315.

Hermansson, A.-M., Svegmark, K. (1996) Developments in the understanding of starch functionality *Trends Food Sci. Technol.*, 7, 345–353.

Hizukuri, S. (1985) Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules *Carbohydr. Res.*, 141, 295–306.

Hizukuri, S. (1986) Polymodal distribution of the chain lengths of amylopectins, and its significance *Carbohydr. Res.*, 147, 342–347.

Inouchi, N., Glover, D.V., Fuwa, H. (1987) Chain length distribution of amylopectins of several single mutants and the normal counterpart, and sugary-1 phytoglycogen in maize (*Zea mays* L.) *Starch/Stärke*, 39, 259–266.

Kalichevsky, M.T., Orford, P.D., Ring, S.G. (1990) The retrogradation and gelation of amylopectins from various botanical sources *Carbohydr. Res.*, 198, 49–55.

Karlsson, R., Olered, R., Eliasson, A.-C. (1983) Changes in starch granule size distribution and starch gelatinization properties during development and maturation of wheat, barley and rye *Starch/Stärke*, 35, 335–340.

Katz, J.R. (1934) X-ray investigations of gelatinization and retrogradation of starch in its importance for bread research *Baker's Weekly*, 81, 34–46.

Katz, J.R., van Itallie, T.B. (1930) Abhandlungen zur physikalischen Chemie der Stärke und der Brotbereitung *Z. Physl. Chem. A.*, 150, 90–99.

Knutson, C.A. (1990) Annealing of maize starches at elevated temperatures *Cereal Chem.*, 67, 376–384.

Krog, N., Nybo-Jensen, B. (1970) Interaction of monoglycerides in different physical states with amylose and their anti-firming effects in bread *J. Food Technol.*, 5, 77–87.

Lineback, D.R. (1984) The starch granule, organization and properties *Baker's Dig.*, 58, 16–21.

Lloyd, J.R., Hedley, C.L., Bull, V.J., Ring, S.G. (1996) Determination of the effect of *r* and *rb* mutations on the structure of amylose and amylopectin in pea (*Pisum sativum* L.) *Carbohydr. Polym.*, 29, 45–49.

MacGregor, A. W., Fincher, G. B. (1993). Carbohydrates of the barley grain. In A. W. MacGregor, R. S. Bhatty, (Eds.), *Barley Chemistry and Technology* (p. 30). St Paul, MI, USA: American Association of Cereal Chemists.

McDonald, A.M.L., Stark, J.R. (1988) A critical examination of procedures for the isolation of barley starch *J. Inst. Brew.*, 94, 125–132.

Meredith, P., Dengate, H.N., Morrison, W.R. (1978) The lipids of various sizes of wheat starch granules *Starch/Stärke*, 30, 119–125.

Miles, M.J., Morris, V.J., Orford, P.D., Ring, S.G. (1985) The roles of amylose and amylopectin in the gelation and retrogradation of starch *Carbohydr. Res.*, 135, 271–281.

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Molecular weights and gyration radii of amylopectins determined by high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors☆

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Abstract

High-performance size-exclusion chromatography (HPSEC) equipped with multi-angle laser-light scattering (MALLS) and refractive index (RI) detectors was used to determine weight-average molecular weight (M_w) and z-average radius of gyration (R_z) of amylopectin of selected starches. Ranges of M_w and R_z values of amylopectin were 7.0×10^7–5.7×10^8 g/mol and 191–782 nm, respectively. Amylopectins of waxy starches had substantially larger M_w than did those of normal starch counterparts. Based on the dispersed-molecular density (M_w/R_z^3), waxy amylopectins displayed, in general, larger dispersed-molecular density than did normal amylopectin counterparts, and amylopectins of the A-type starches had larger dispersed-molecular density than did those of the B-type starches. These results suggested that amylopectins of waxy starches had more branch-chains and no extra long chains, which resulted in more densely packed molecules than did those of normal starch counterparts. The amylopectin of B-type starch had longer but fewer branch-chains, which resulted in smaller dispersed density than did that of the A-type starch. M_w and R_z values of amylose isolated from amylomaize VII starch were also determined to be 2.8×10^5 and 43 nm, respectively.

Keywords: Amylopectin; Weight-average molecular weight (M_w); z-Average radius of gyration (R_z); Multi-angle laser-light scattering (MALLS)

1. Introduction

Starch is one of the most important biopolymers. There are two major components of starch: amylopectin that is a highly branched gigantic molecule and amylose, a primarily linear molecule. Functional properties of starch are affected by molecular weight of amylose and amylopectin. Larger molecular weight (DP_n) of amylose and amylopectin resulted in higher pasting peak viscosity in wheat (Shibanuma, Takeda, & Hizukuri, 1996) and sago starches (Takeda, Takeda, Suzuki, & Hizukuri, 1989). Jane and Chen (1992) reported that the long branch chain-length of amylopectin and the intermediate size of amylose produced the greatest synergistic effect on pasting viscosity of reconstituted starch.

Branch structures of amylopectin molecules have been studied using various chromatographic techniques such as gel permeation chromatography (Craig & Stark, 1984; Jane & Chen, 1992; Wang, White, Pollak, & Jane, 1993), high-performance size-exclusion chromatography (Hizukuri, 1985; Ong, Jumel, Tokarczuk, Blanshard, & Harding, 1994; Yuan, Thompson, & Boyer, 1993), and high-performance anion-exchange chromatography (Jane et al., 1999; Koizumi, Fukuda, & Hizukuri, 1991; Wong & Jane, 1997). Determination of amylopectin molecular weight is challenging because of its gigantic molecules, which are larger than any other synthetic and natural polymers. Lack of calibration standards causes difficulties in determination of the molecular weight of amylopectin using size-exclusion chromatography.

HPSEC equipped with multi-angle laser-light scattering (MALLS) and refractive index (RI) detectors has been applied to determine absolute molecular weight of starches (Aberle, Burchard, Vorwerg, & Radosta, 1994; Fishman, Rodriguez, & Chau, 1996). The MALLS detection technique combining with HPSEC is a powerful tool for determining the absolute molecular weights of such macromolecules. To obtain an accurate molecular weight of amylopectin

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using this technique, complete separation of amylopectin from amylose is required.

Methods used to disperse starch molecules for chromatography are critical because entangled amylose/amylopectin molecules cannot be separated by SEC and will affect the molecular weight and gyration radius determined by the MALLS technique. It has been reported that total starch solubility increased with increasing proportion of amylose (Jackson, 1991). Waxy-type starches, on the other hand, are more susceptible to shear-induced fragmentation than are normal starches (Bello-Pérez, Roger, Baud, & Colonna, 1998; Hanselmann, Ehrat, & Widmer, 1995; Millard, Dintzis, Willett, & Klavons, 1997). M_w of waxy maize was reported to be 6.5×10^7 or 5.9×10^8 depending on dispersing conditions (Millard et al., 1997). Jackson (1991) studied the extent of solubility of maize starches in dimethyl sulfoxide (DMSO) using various conditions. He confirmed that maximum dispersibility was achieved using a solution of 90% DMSO/10% water solution. In this study, we used a gentle procedure to prepare starch dispersion in an aqueous solution containing 90% DMSO and separated amylopectin from amylose using an HPSEC system. Molecular weights and gyration radii of amylopectins were determined by using on-line MALLS and RI detection. Branch structures of amylopectin molecules were proposed to explain the relationship between the molecular weights and gyration radii obtained for starches of A- and B-type polymorphisms.

2. Materials and methods

2.1. Materials

Chinese taro, mung bean, waxy rice, sweet rice, green leaf canna, lotus root, water chestnut, cattail millet, normal and waxy barley (from Dr C. W. Newman), and waxy wheat (from Dr R. A. Graybosch) starches were isolated in our laboratory. Other starches were gifts from Dr A. R. Bonilla (green banana), Cerestar, USA (waxy, *ae*, and *du* waxy maize), National Starch and Chemical (tapioca, normal maize, and amylomaize V and VII), and Lykkeby Starkelsen Food and Fiber AB (waxy potato). Normal rice (from Matheson Coleman and Bel), normal potato and wheat (from Sigma Chemical) starches were purchased. Pullulan standards (Shodex STANDARD P-82) were purchased from Showa Denko K.K. (Tokyo, Japan). Isoamylase (EC 3.2.1.68) from *Pseudomonas amyloderamosa* was purchased from Hayashibara Biochem. Lab. (Okayama, Japan). Deionized water (18.2 MΩ cm) used as an eluent and for sample preparation was obtained from a Milli-Q Reagent Water System (Millipore, Bedford, MA). Other chemicals were reagent grade and used without further treatment.

2.2. Preparation of starch aqueous dispersions for HPSEC

Starch (120 mg) was evenly wetted with 1.2 ml of water and then dispersed in 10.8 ml of dimethyl sulfoxide (DMSO). The suspension was mechanically stirred while heating in a boiling water bath for 1 h and then stirred for 24 h at 25°C. An aliquot (0.4 ml) of starch dispersion (1.0%, w/v) was mixed with five volume of ethanol (2 ml) to precipitate starch. Ethanol-precipitated starch was separated by centrifugation at 6750 × g for 20 min. The starch pellet was then redissolved in boiling water (10 ml) and stirred for 30 min in a boiling water bath. The hot sample solution was filtered through a nylon membrane filter (5.0 μm) and then injected into an HPSEC system. The final concentration of the starch solution filtrate injected (100 μl) was 0.4 mg/ml.

2.3. Molecular weight distribution of amylopectin determined by an HPSEC-MALLS-RI system

An HPSEC system consisted of an HP 1050 isocratic pump (Hewlett Packard, Valley Forge, PA) equipped with an injection valve (100 μl sample loop, Model 7125, Rheodyne), a multi-angle laser-light-scattering detector (Dawn DSP-F, Wyatt Tech. Corp., Santa Barbara, CA) with a He–Ne laser source ($\lambda = 632.8$ nm) and a K-5 flow cell, and an HP 1047A RI detector (Hewlett Packard, Valley Forge, PA). To separate amylopectin from amylose, Shodex OH pak KB-G guard column and KB-806 and KB-804 analytical columns (Showa Denko K.K., Tokyo, Japan) were used. The temperature of the injector and columns was maintained at 55.0°C using a CH-460 column heater and a TC-50 controller (Eppendorf, Madison, WI). Temperature of RI detector was set at 30.0°C. The mobile phase was distilled–deionized water (18.2 MΩ cm) passed through in-line membrane filters (0.2 and 0.1 μm, Millipore, Bedford, MA) at a flow rate of 0.7 mL/min.

2.4. Data analysis

A pullulan standard, P-20 ($M_w = 2.28 \times 10^6$, 5 mg/ml), was used for normalization of multiangle photodiode detectors and for determination of delay volume (0.222 ml) between MALLS and RI detections. Data obtained from MALLS and RI detectors were analyzed using Astra software (Version 4.7.07, Wyatt Technology, Santa Barbara, CA). M_w was calculated using the following equation:

$$K^*c/R_\theta = 1/[M_w P(\theta)] - 2A_2 c \quad (1)$$

where R_θ is the excess intensity of scattered light at angle θ, c the sample concentration, M_w the weight-average molecular weight, A_2 a second viral coefficient; K^* an optical parameter equal to $4\pi^2 n_0^2 (dn/dc)^2/(\lambda_0^4 N_A)$ where n_0 is the solvent RI and dn/dc is the RI increment, N_A Avogadro's number and λ_0 is the wavelength of the scattered light in vacuum. Function $P(\theta)$ describes the angular dependence of scattered light. Expansion of $1/P(\theta)$ to first order gives:

$$1/P(\theta) = 1 + (16\pi^2/3\lambda^2)\langle r_g^2 \rangle \sin^2(\theta/2) + f_1 \sin^4(\theta/2) + \cdots \quad (2)$$

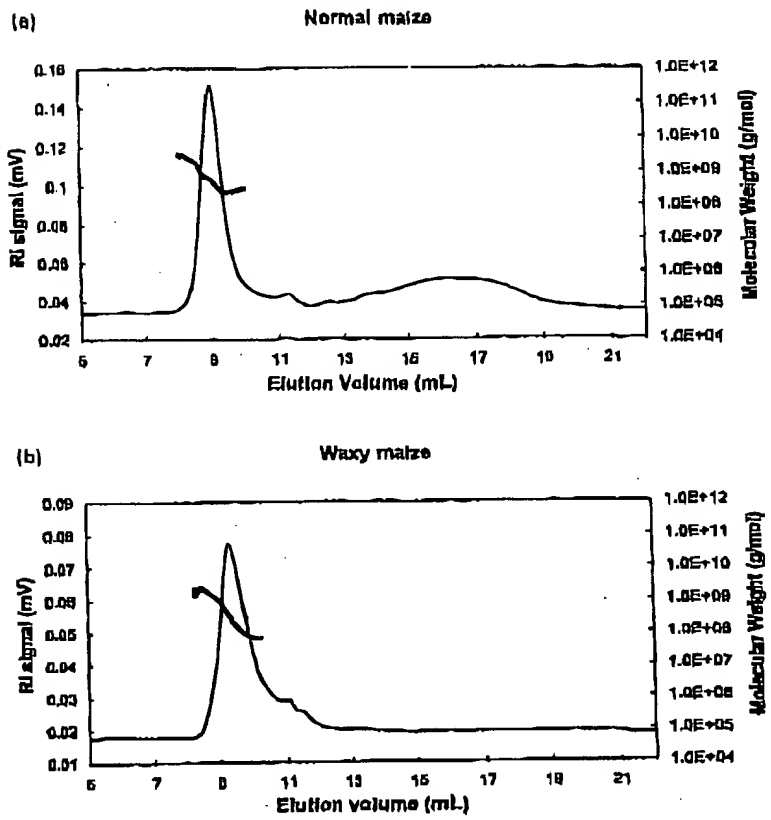

Fig. 1. Amylopectin molecular weight distributions (—) of maize (A) and waxy maize (B) determined by an HPSEC–MALLS–RI system. The RI signal profile (—) is shown throughout the elution volume.

Curve fitting method in this study was based on a second order Berry method ($\sqrt{(K^*c/R_\theta)}$ vs. $\sin^2(\theta/2)$), and a second virial coefficient, A_2 was set at zero (Wen, Arakawa, & Philo, 1996; Yokoyama, Renner-Nantz, & Shoemaker, 1998). M_w and R_z (equal to r_g in Eq. (2)) values were calculated from intercept and slope of the Eq. (1) by extrapolating multiangle signals to zero angle, respectively. The dn/dc value of 0.146 ml/g was used in this calculation (Bello-Pérez et al., 1998; Fishman et al., 1996; Roger & Colonna, 1993).

3. Results and discussion

Molecular weight distributions of amylopectins of normal and waxy maize starches, eluted by distilled–deionized water as the mobile phase and traced by an RI detector, are shown as examples in Fig. 1A and B, respectively. A good separation of amylopectin from amylose was achieved by using the HPSEC system with the operating conditions reported in materials and methods, which enabled us to conduct accurate measurements of amylopectin molecular weights (Fig. 1A). Weight-average molecular weights (M_w) of amylopectins of different starches, calculated using a second order Berry method, are shown in Table 1. Molecular weights and gyration radii of amylopectins obtained in this study were larger than those reported in other studies (Aberle et al., 1994; Fishman et al., 1996). Differences between these results and others can be attributed to the methods of preparing starch dispersions. Millard et al. (1997) reported that methods used to prepare starch dispersion significantly affected molecular weight determination. Using a mild condition, such as direct dispersion in 90% DMSO, they reported M_w of waxy maize starch to be 7.5×10^8 g/mol, which was in agreement with our result (8.3×10^8 g/mol). Millard, Wolf, Dinzis, and Willett (1999) dispersed starch in 90% DMSO and compared the M_w of waxy maize starch obtained from static light scattering with that obtained from analytical ultracentrifugation. In their study, the M_w analyzed by latter method (5.93×10^8 g/mol), calculated using the Svedberg equation, was in good agreement with the light scattering result (5.60×10^8 g/mol). The same authors also reported R_z value (342 nm) for waxy maize starch, which was comparable to R_z (372 nm) calculated from our result.

Another important factor affecting molecular weight

Table 1
Amylopectin molecular weights and gyration radii of selected starches (data were averages of at least two injections)

	M_w ($\times 10^8$)[a]	R_z (nm)[b]	ρ (g/mol/nm^3)[c]
A-type starches			
Normal maize	4.9 (0.8)[d]	312 (23)	16.1
Waxy maize	8.3 (0.2)	372 (11)	16.1
du wx maize	4.9 (0.5)	312 (13)	16.1
Normal rice	26.8 (2.9)	581 (41)	13.7
Waxy rice	56.8 (9.3)	782 (36)	11.9
Sweet rice	13.9 (1.0)	486 (5)	12.1
Normal wheat	3.1 (0.3)	302 (3)	11.3
Waxy wheat	5.2 (0.4)	328 (6)	14.7
Barley	1.3 (0.1)	201 (8)	16.0
Waxy barley	6.8 (0.1)	341 (3)	17.1
Cattail millet	2.7 (0.2)	278 (6)	12.6
Mung bean	3.8 (0.2)	312 (3)	12.5
Chinese taro	12.6 (3.6)	560 (15)	7.2
Tapioca	0.7 (0.1)	191 (25)	10.0
B-type starches			
ae wx maize	3.2 (0.2)	306 (8)	11.2
Amylomaize V	2.4 (0.0)	357 (24)	5.3
Amylomaize VII	1.7 (0.0)	389 (57)	2.9
Potato	1.7 (0.2)	356 (36)	3.8
Waxy potato	2.0 (0.2)	344 (37)	4.9
Green leaf canna	3.4 (2.2)	436 (85)	4.1
C-type starches			
Lotus root	1.5 (0.4)	280 (57)	6.8
Water chestnut	7.1 (1.5)	230 (25)	58.4
Green banana	1.9 (0.8)	286 (29)	8.1
Glycogen			
Cyanobacterial glycogen[e]	0.2 (0.0)	55 (4)	99.2

[a] Weight-average molecular weight.
[b] z-average radius of gyration.
[c] Density (ρ) = M_w/R_z^3.
[d] Standard deviation.
[e] Glycogen was isolated from *Synechocystis* sp. PCC6803 in our laboratory.

calculation is the data fitting method. Yokoyama et al. (1998) applied three different methods (Berry, 1966; Debye, 1947; Zimm, 1948) to analyze M_w and R_z. The authors found that the M_w calculated by the Zimm method was significantly larger than that calculated by Berry and Debye methods. The Zimm method can yield unreasonable results (Aberle et al., 1994), but the Berry method is demonstrated to determine M_w of larger molecules with greater accuracy (Hanselmann et al., 1995). Thus, we chose the second order Berry method (Millard et al., 1997) for our study. The second-order Berry method gave a good curve fitting with laser signals obtained at different angles as shown in amylopectin of sweet rice starch at the peak of the RI signal (Fig. 2).

Among the M_w of the amylopectins reported in Table 1, amylopectins of waxy starches consistently displayed larger M_w than did those of normal starch counterparts. Carbon flux at a form of ADP-Glc (adenosine-5′-diphosphate glucose) is partitioned between amylose and amylopectin in normal starch biosynthesis. Granule-bound starch synthase I (GBSSI) is primarily involved in amylose biosynthesis of starch (Buléon, Colonna, Planchot, & Ball, 1998; Smith, Denyer, & Martin, 1997). In waxy mutants, GBSSI is missing and no amylose is synthesized. It is plausible that ADP-Glc is exclusively incorporated into amylopectin molecules resulting in amylopectin molecules with larger M_w in waxy mutants. Carbon partitioning could also explain substantially smaller M_w of amylopectins of amylomaize V and VII than those of normal and *ae wx* maize. Amylomaize VII has been reported to have less amylopectin of large molecular weight than does normal maize amylopectin revealed by HPSEC (Takeda, Takeda, & Hizukuri, 1993). Fishman et al. (1996) also found, in the analysis of waxy, normal, amylomaize V, and amylomaize VII starches, that the molecular weight of amylopectin decreased with the increase in the amylose content in maize starch. It can also be postulated that space limitation in the normal starch granule because of the presence of ca. 25% by mass of amylose molecules results in smaller M_w of normal amylopectin.

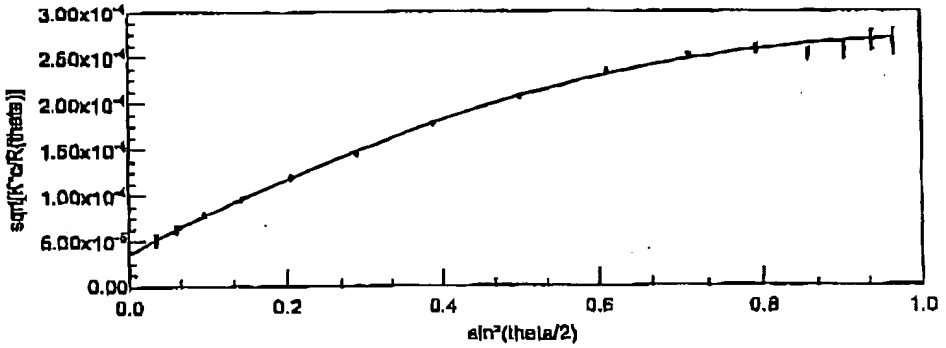

Fig. 2. Light scattering data plot ($\sqrt{(K^*c/R_\theta)}$ vs. $\sin^2(\theta/2)$) of sweet rice amylopectin at the peak concentration based on the second order Berry method. Three detectors at the lowest angle were not used for the curve fitting.

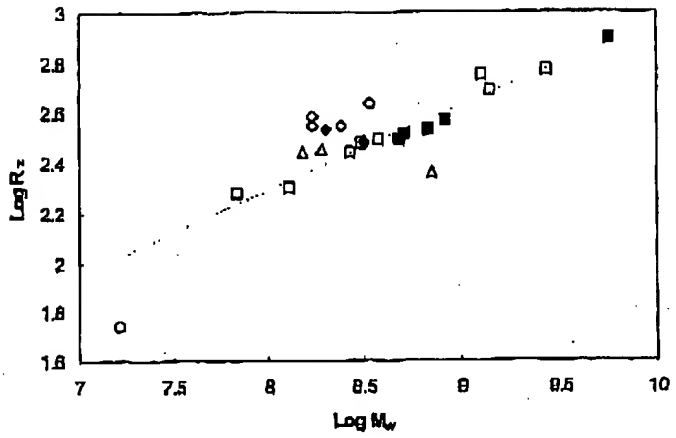

Fig. 3. Relationships of amylopectins between the weight-average molecular weight (M_w) and z-average radius of gyration (R_z). Data are plotted on Log–Log scale: A-type (□); B-type (◇); C-type (△); waxy A-type (■); waxy B-type (◆) amylopectins; glycogen (○). The linear regression line on the graph comprises data of A-type amylopectin.

The M_w of amylopectins isolated from A-type starches varied to a larger range ($0.7–56.8 \times 10^8$) than did those isolated from B-type starches ($1.7–3.4 \times 10^8$). Many amylopectins of A-type starches had larger M_w than did those of B-type starches. Amylopectins of B-type starches also had larger R_z than did those of A-type starches having comparable molecular weights (Fig. 3). Because R_z is related to the volume occupied by the molecule in a solution (Millard et al., 1997), the branch chain-length and branching pattern of the amylopectin molecule are expected to affect the R_z of amylopectin in the solution. Glycogen, being highly branched and compact molecules, displayed a substantially larger dispersed-molecular density ($\rho = M_w/R_z^3$) than did amylopectin (Table 1).

When the log R_z was plotted against log M_w of amylopectin (Fig. 3), A-type starches showed a linear relationship with a slope of 0.334 and a correlation coefficient of $r = 0.98$ ($P < 0.05$). The strong linear relationship between log R_z and log M_w was likely due to similar branching structures of amylopectins among A-type starches. The slope (0.334) indicated that the A-type amylopectins have highly compact spherical forms (Wyatt, 1993). Data of the B-type amylopectins, however, did not give a linear relationship, which could be attributed to differences in branch structures

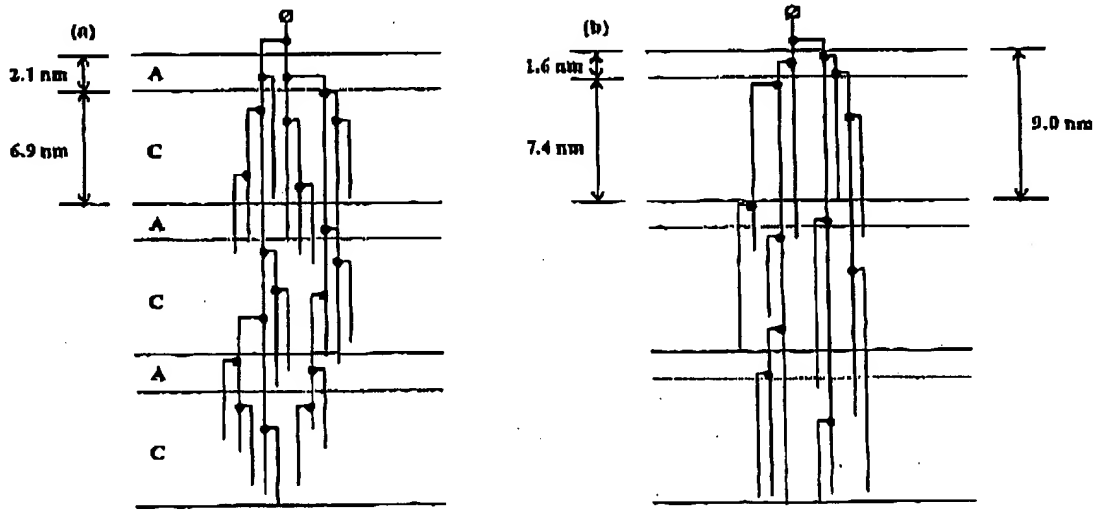

Fig. 4. Structure models of amylopectins of a, normal maize (A-type) and b, amylomaize VII (B-type) starches. A and C stand for the amorphous and crystalline regions, respectively. A repeating distance of 9.0 nm for the cluster (Jenkins et al., 1993; Jenkins & Donald, 1995) and A:B chain ratio of 1.2:1 (Yun & Matheson, 1993) for both starches are used for the models. Average branch chain-lengths of normal maize and amylomaize VII amylopectins are 24 and 31, respectively (Jane et al., 1999).

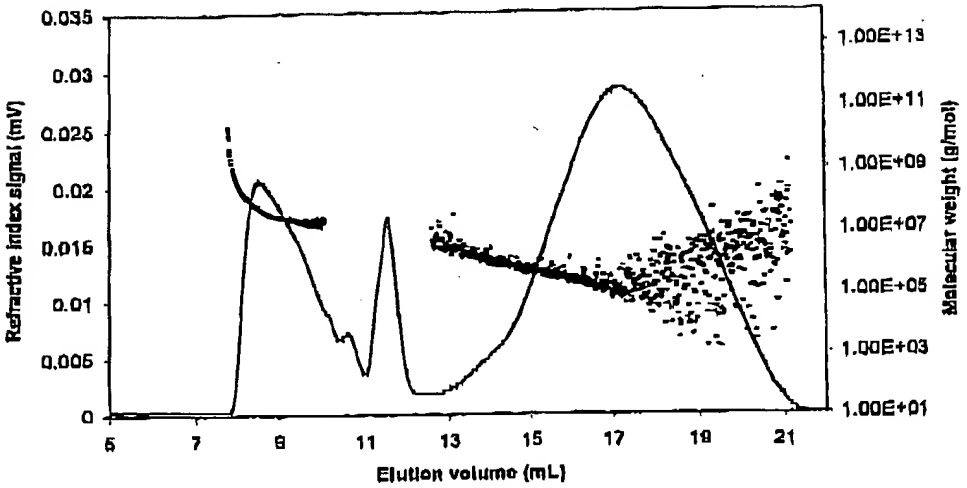

Fig. 5. Molecular weight distribution (——) of amylomaize VII starch determined by an HPSEC–MALLS–RI system. The RI signal profile (—) is shown throughout the elution volume.

of B-type amylopectins. Low density of dispersed B-type amylopectin molecules could result in undefined conformation in aqueous solutions.

Most amylopectins of waxy starches displayed larger dispersed-molecular densities than those of normal starch counterparts (Table 1). The difference might be attributed to extra-long chains of amylopectins. Extra-long chains (ELC), branch-chains that have structures resembling amylose (DP770 or larger) and carry few branches, were found in normal amylopectin but not in waxy amylopectin (Takeda & Hizukuri, 1987; Takeda, Shitaozono, & Hizukuri, 1988; Yoo & Jane, submitted). The presence of ELC that carry few branches in normal amylopectin is likely to decrease the dispersed-molecular density of normal amylopectin. The ELCs that carry few branches can also contribute to the smaller M_w of normal amylopectin. Two A-type amylopectins that displayed smaller dispersed-molecular densities were Chinese taro and tapioca; both are root starches.

Molecular densities of dispersed B-type amylopectins were all smaller than those of the A-type amylopectins except *ae wx* amylopectin (Table 1). Amylopectins of B-type starches comprise longer branch-chains and larger proportions of long B chains than do those of the A-type starches (Hizukuri, 1996; Jane et al., 1999; Takeda et al., 1993). Hizukuri (1985) reported a ratio of short chains to long chains of normal maize amylopectin to be 10, which was at least three times larger than the ratio (3) for amylomaize amylopectin. Differences in branch structures between the A-type and the B-type starches were proposed by Jane, Wong, and McPherson, (1997). They suggest that there are more branches in A-type amylopectins and branch linkages of the A-type amylopectin are scattered and located both in the amorphous and the crystalline regions, whereas there are fewer branches in the B-type amylopectins and most branch linkages are clustered in the amorphous region.

The differences in the molecular weight, gyration radius, and dispersed-molecular density of the A- and B-type amylopectin can be explained by molecular structures of the respective molecules. Structural models constructed using parameters of repeating distance of 9.0 nm (Jenkins & Donald, 1995; Jenkins, Cameron, & Donald, 1993), A/B chain ratio of 1.2:1 (Yun & Matheson, 1993), and the chain-length distribution and molar chain ratio (Jane et al., 1999) for both normal maize (A-type) and amylomaize VII (B-type) amylopectins are shown in Fig. 4. Amylomaize VII has much longer exterior chains (Cheetham & Tao, 1997; Takeda et al., 1993) and larger long-chain/short-chain ratios (Hizukuri, 1985; Jane et al., 1999) than does normal maize. The structure models clearly demonstrate the larger dispersed-molecular density of the A-type amylopectin than that of the B-type.

It was difficult to calculate M_w and R_z of amylose by using the same on-line HPSEC–MALLS–RI system. With the sample concentration of 0.4 mg/ml used in this study and amylose molecules being highly polydispersed, we could not obtain sufficient laser-light scattering signals to calculate molecular weight distributions of amylose of most starch samples. We could, however, determine M_w and R_z of amylose of amylomaize VII that contained about 70% of apparent amylose. The weight-average molecular weight (M_w) and z-average radius of gyration (R_z) of amylose of amylomaize VII were 2.8×10^5 and 43 nm, respectively. The molecular weight was similar to the M_w (1.8×10^5) of amylose calculated by Fishman et al. (1996) using HPSEC-viscometry (Fishman & Hoagland, 1994). Suortti, Gorenstein, and Roger (1998) reported the M_w of amylose to be $4.0–5.0 \times 10^5$ using laser-light scattering technique. The molecular weight distribution of amylomaize VII is shown

in Fig. 5. The weight-average molecular weight of amylose was calculated by using first order Zimm method. The amyloses of amylomaize V and VII have been reported to have the smallest molecular weights among all starches studied (Hizukuri, 1996; Jane & Chen, 1992).

4. Conclusion

Molecular weights (M_w) of amylopectins varied from 7.0×10^7 to 5.7×10^9, depending on the botanical source. Amylopectins of waxy starches had larger molecular weights, and most had larger dispersed-molecular densities than did those of normal amylopectin counterparts. The M_w of A-type amylopectins varied to a larger range (7.0×10^7 to 5.7×10^9) than did that of B-type amylopectins ($1.7–3.4 \times 10^8$). At the same molecular weight, amylopectins of B-type starches had larger R_z than did those of A-type starches. Different branch structures of amylopectins of A- and B-type starches resulted in different dispersed-molecular densities in dilute solutions. The M_w and R_z of amylose from amylomaize VII were 2.8×10^5 and 43 nm, respectively.

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References

Aberle, T., Burchard, W., Vorwerg, W., & Radosta, S. (1994). Conformational contributions of amylose and amylopectin to the structural properties of starches from various sources. *Starch/Staerke, 46*, 329–335.

Bello-Pérez, L. A., Roger, P., Baud, B., & Colonna, P. (1998). Macromolecular features of starches determined by aqueous high-performance size exclusion chromatography. *Journal of Cereal Science, 27*, 267–278.

Berry, G. C. (1966). Thermodynamic and conformational properties of polystyrene I. Light-scattering studies on dilute solutions of linear polystyrenes. *Journal of Chemical Physics, 44*, 4550–4564.

Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules, 23*, 85–112.

Cheetham, N. W. H., & Tao, L. (1997). Amylose conformational transitions in binary DMSO/water mixtures. *Starch/Staerke, 49*, 407–415.

Craig, S. A. S., & Stark, J. R. (1984). A comparison of the molecular properties of sorghum starches of different origins. *Starch/Staerke, 36*, 127–131.

Debye, P. (1947). Molecular-weight determination by light scattering. *Journal of Physical Colloidal Chemistry, 51*, 18–32.

Fishman, M. L., & Hoagland, P. D. (1994). Characterization of starches dissolved in water by microwave heating in a high pressure vessel. *Carbohydrate Polymer, 23*, 175–183.

Fishman, M. L., Rodriguez, L., & Chau, H. K. (1996). Molar masses and sizes of starches by high-performance size exclusion chromatography with on-line multiangle laser light scattering detection. *Journal of Agriculture Food Chemistry, 44*, 3182–3188.

Hanselmann, R., Ehret, M., & Widmer, H. M. (1995). Sedimentation field flow fractionation combined with multi angle laser light scattering applied for characterization of starch polymers. *Starch/Staerke, 46*, 345–349.

Hizukuri, S. (1985). Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydrate Research, 141*, 295–306.

Hizukuri, S. (1996). Starch: analytical aspect in carbohydrates in food. In A. Eliasson, *carbohydrates in food* (pp. 392–393). N.Y: Marcel Dekker.

Jackson, D. S. (1991). Solubility behavior of granular corn starches in methyl sulfoxide (DMSO) as measured by high performance size exclusion chromatography. *Starch/Staerke, 43*, 422–427.

Jane, J., & Chen, J. (1992). Effect of amylose molecular size and amylopectin branch chainlength on paste properties of starch. *Cereal Chemistry, 69*, 60–65.

Jane, J., Wong, K. S., & McPherson, A. E. (1997). Branch-structure difference on starches of A- and B-type X-ray patterns revealed by their Naegeli dextrins. *Carbohydrate Research, 300*, 219–227.

Jane, J., Chen, Y. Y., Lee, L. F., McPherson, A. E., Wong, K. S., Radosavljevic, M., & Kasemsuwan, T. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry, 75*, 629–637.

Jenkins, P. J., & Donald, A. M. (1995). The influence of amylose on starch granule structure. *International Journal of Biological Macromolecules, 17*, 315–321.

Jenkins, P. J., Cameron, R. E., & Donald, A. M. (1993). A universal feature in the structure of starch granules from different botanical sources. *Starch/Staerke, 45*, 417–420.

Koizumi, K., Fukuda, M., & Hizukuri, S. (1991). Estimation of the distributions of chain length of amylopectins by high-performance liquid chromatography with pulsed amperometric detection. *Journal of Chromatography, 585*, 233–238.

Millard, M. M., Dintzis, F. R., Willett, J. L., & Klavons, J. A. (1997). Light-scattering molecular weights, intrinsic viscosities of processed waxy maize starches in 90% DMSO and H_2O. *Cereal Chemistry, 74*, 687–691.

Millard, M. M., Wolf, W. J., Dintzis, F. R., & Willett, J. L. (1999). The hydrodynamic characterization of waxy maize amylopectin in 90% dimethyl sulfoxide–water by analytical ultracentrifugation, dynamic, and static light scattering. *Carbohydrate Polymer, 39*, 315–320.

Ong, M. H., Jumel, K., Tokarczuk, P. F., Blanshard, J. M. V., & Harding, S. E. (1994). Simultaneous determinations of the molecular weight distributions of amyloses and the fine structures of amylopectins of native starches. *Carbohydrate Research, 260*, 99–117.

Roger, P., & Colonna, P. (1993). Evidence of the presence of large aggregates contamination amylose solutions. *Carbohydrate Polymer, 21*, 83–89.

Shibanuma, Y., Takeda, Y., & Hizukuri, S. (1996). Molecular and pasting properties of some wheat starches. *Carbohydrate Polymer, 29*, 253–261.

Smith, A. M., Denyer, K., & Martin, C. (1997). The synthesis of the starch granule. *Annual Review of Plant Physiology and Plant Molecular Biology, 48*, 67–87.

Suortti, T., Gorenstein, M. V., & Roger, P. (1998). Determination of the molecular mass of amylose. *Journal of Chromatography, A, 828*, 515–521.

Takeda, Y., & Hizukuri, S. (1987). Structures of rice amylopectins with high and low affinities for iodine. *Carbohydrate Research, 168*, 79–88.

Takeda, Y., Shitaozono, T., & Hizukuri, S. (1988). Molecular structure of corn starch. *Starch/Staerke, 40*, 51–54.

Takeda, Y., Takeda, C., Suzuki, A., & Hizukuri, S. (1989). Structures and properties of sago starches with low and high viscosities on amylography. *Journal of Food Science, 54*, 177–182.

Takeda, C., Takeda, Y., & Hizukuri, S. (1993). Structure of the amylopectin fraction of amylomaize. *Carbohydrate Research, 246*, 173–281.

Wang, Y. -J., White, P., Pollak, L., & Jane, J. (1993). Characterization of starch structures of 17 maize endosperm mutant genotypes with Oh43 inbred line background. *Cereal Chemistry, 70*, 171–179.

Wen, J., Arakawa, T., & Philo, J. S. (1996). Size-exclusion chromatography with on-line light-scattering, absorbance, and refractive index detectors for studying proteins and their interactions. *Analytical Biochemistry, 240*, 155–166.

Wong, K. S., & Jane, J. (1997). Quantitative analysis of debranched amylopectin by HPAEC-PAD with a post-column enzyme reactor. *Journal of Liquid Chromatography, 20*, 297–310.

Wyatt, P. J. (1993). Light scattering and the absolute characterization of macromolecules. *Analytica Chimica Acta, 272*, 1–40.

Yokoyama, W., Renner-Nantz, J. J., & Shoemaker, C. F. (1998). Starch molecular mass and size by size exclusion chromatography in DMSO–LiBr coupled with multiple angle laser light scattering. *Cereal Chemistry, 75*, 530–535.

Yoo, S. -H., & Jane, J., Structural and physical characteristics of waxy and other wheat starches. Carbohydrate Polymers, submitted for publication.

Yuan, R. C., Thompson, D. B., & Boyer, C. D. (1993). Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from the waxy-containing genotypes in two inbred lines. *Cereal Chemistry, 70*, 81–89.

Yun, S. -H., & Matheson, N. K. (1993). Structures of the amylopectins of waxy, normal, amylose-extender, and waxy genotypes and of the phytoglycogen of maize. *Carbohydrate Research, 243*, 307–321.

Zimm, B. H. (1948). The scattering of light and the radial distribution function of high polymer solutions. *Journal of Chemistry and Physics, 16*, 1093–1099.

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Session I: Structure of Starch and Its Biosynthesis

Structures of Amylopectin and Starch Granules: How Are They Synthesized ?

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Key words: starch, amylopectin, granule structure, enzyme degradation

Starch is synthesized by higher plants in nature and is produced as semi crystalline granules. Starch granules consist of highly branched amylopectin molecules with their external branch chains folded in double helical crystalline structure and primarily linear amylose molecules present in amorphous form. Consequently, waxy starch that consists of pure amylopectin displays a greater degree of crystallinity than the normal starch counterpart that consists of about 25% amylose. Because of the partially crystalline structure, starch granules are insoluble in water at temperatures below the gelatinization temperature of the starch.[1] Under a polarized light microscope, starch granules display Maltese-cross patterns, indicating radial arrangements of amylose and amylopectin molecules in the granule. Amylose and amylopectin molecules in the granule are synthesized by apposition and oriented perpendicular to the surface of starch granules.

Relative locations of amylose and amylopectin molecules in the starch granule.

To investigate if amylose molecules are interspersed among amylopectin molecules or are present in bundles, chemical cross-linking reactions were applied to intact native starch granules. Results of the cross-linking reaction studies show that amylose molecules are cross-linked onto amylopectin molecules and amylopectin molecules are also cross-linked among themselves. No cross-links are found, however, between amylose molecules.[2,3] These results indicate that amylose molecules are interspersed among amylopectin instead of being present in bundles, as speculated for amylose based on its escaping from branching enzyme reactions. The fact that amylose and amylopectin molecules are interspersed in the granule and amylose is not subjected to the branching enzyme reaction, the mechanism can now be explained on the basis that the two molecules are synthesized side by side by two different starch synthases, granular bound starch synthase[4] and soluble starch synthase,[5] respectively.[6,7]

Is the internal structure of the starch granule homogeneous ?

To find out if structures and compositions of amylose and amylopectin are the same through out the granule (*e.g.*, at the hilum and on the periphery), which are synthesized at different time order, Jane and Shen[8] peeled starch granules by layers to determine the structure at different radial locations. The authors used a surface gelatinization technique by treating native starch granules with saturated neutral salt solution, such as LiCl and $CaCl_2$, for different time periods to achieve different surface gelatinization percentages. Using this method, Jane and Shen[8] and Pan and Jane[10] reported that amylose is more concentrated on the periphery of the starch granule than at the core of the granule. This is consistent with the observation that starch granules of larger sizes (more mature) contain more amylose than do those of smaller sizes.[9] Branch chains of amylopectin molecules that are located at the core (around the hilum) of the granule are longer than that of the amylopectin located on the periphery. Scanning electron micrographs of the remaining starch granules after a substantial portion (up to 80%) of surface starch has been gelatinized and removed show that starch molecules around the hilum are in loosely packed amorphous structures.[10]

Branch structures of amylopectin: How do they differ between the A- and the B-type crystalline starches ?

It is well established that the branch chain lengths differ between the A-type and the B-type crystalline starches;[11] starch with longer branch chains displays the B-type X-ray diffraction pattern and that with shorter chains displays the A-pattern. Naegeli dextrins prepared by an extensive acid hydrolysis of various native starch granules also show different structures.[12] Naegeli dextrins of starches that display the B-type crystalline pattern consist of mostly linear chains (with a peak DP 13) and few branched chains (peak DP ~25). Whereas, Naegeli dextrins of the A crystalline-type starches consist of a large proportion of singly branched chains with a peak DP 25.[12] The authors interpret the results as that the amylopectin branch linkages of the B crystalline-type starches are more clustered and are located mostly in the amorphous

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region of the amylopectin molecule. Thus, the branch linkages are readily susceptible to acid hydrolysis and are mostly lost during the acid hydrolysis. In contrary, the amylopectin branch linkages of the A crystalline-type starches are scattered in both the amorphous and the crystalline region. Those branch linkages located in the crystalline region are protected from acid hydrolysis, and thus are preserved after the extensive acid treatments.

These proposed structures are in agreement with well-known facts that the A crystalline-type starches possess more short branch-chains and are more easily digestible by amylases than the B crystalline-type starches (Fig. 1). The proposed structures show that the B crystalline-type starches consist of double helices of branch chains that are more orderly aligned in the granule, and thus, they are more resistant to enzyme hydrolysis. The B crystalline-type starch granules (*e.g.*, potato) have a smooth surface without visible pinholes. This is attributed to the lack of weak points in the starch structure. The A crystalline-type starches are easily hydrolyzed by amylases, and the starch granules frequently display pinholes.[12] Animal-feeding studies using maize mutant starches of isogenic background also confirmed that starches consisting of shorter amylopectin branch chains, such as *sugary-2* maize starch, and starches of little or no amylose, such as waxy maize starch, are more easily digestible than the normal starch counter part.[14]

Molecular weights and structures of amylopectin molecules of different origins.

Recent studies using high-performance size-exclusion chromatography have shown that amylopectin molecules of waxy starches display larger molecular weights than those of the normal starch counter parts.[15,16] Amylopectin molecules of the B crystalline-type starches, in general, have smaller molecular weights and lesser densities of dispersed molecules than that of the A crystalline-type crystalline-starches (Table 1 and Fig. 2).[14] The differences can be attributed to the following factors:[15,16]

1) The carbon flux of starch biosynthesis exclusively goes to amylopectin in waxy starch, but a portion (~25 %) of carbon flux goes to the biosynthesis of amylose in normal starch.

2) The A crystalline-type starch amylopectin consists of more short branch chains, which makes the molecules denser at the dispersed form. The B crystalline-type amylopectin carries less B1 chains, but more long chains that increase the gyration radius and result in lesser density of dispersed molecules.

3) Normal maize, barley, wheat, and other cereal amylopectin molecules carry extra-long branch chains,[15,17] but waxy amylopectin counterparts do not. The extra-long, amylose-like chains do not carry many other branch chains like a normal amylopectin branch chain and, thus, can reduce the molecular weight and the dispersed density.

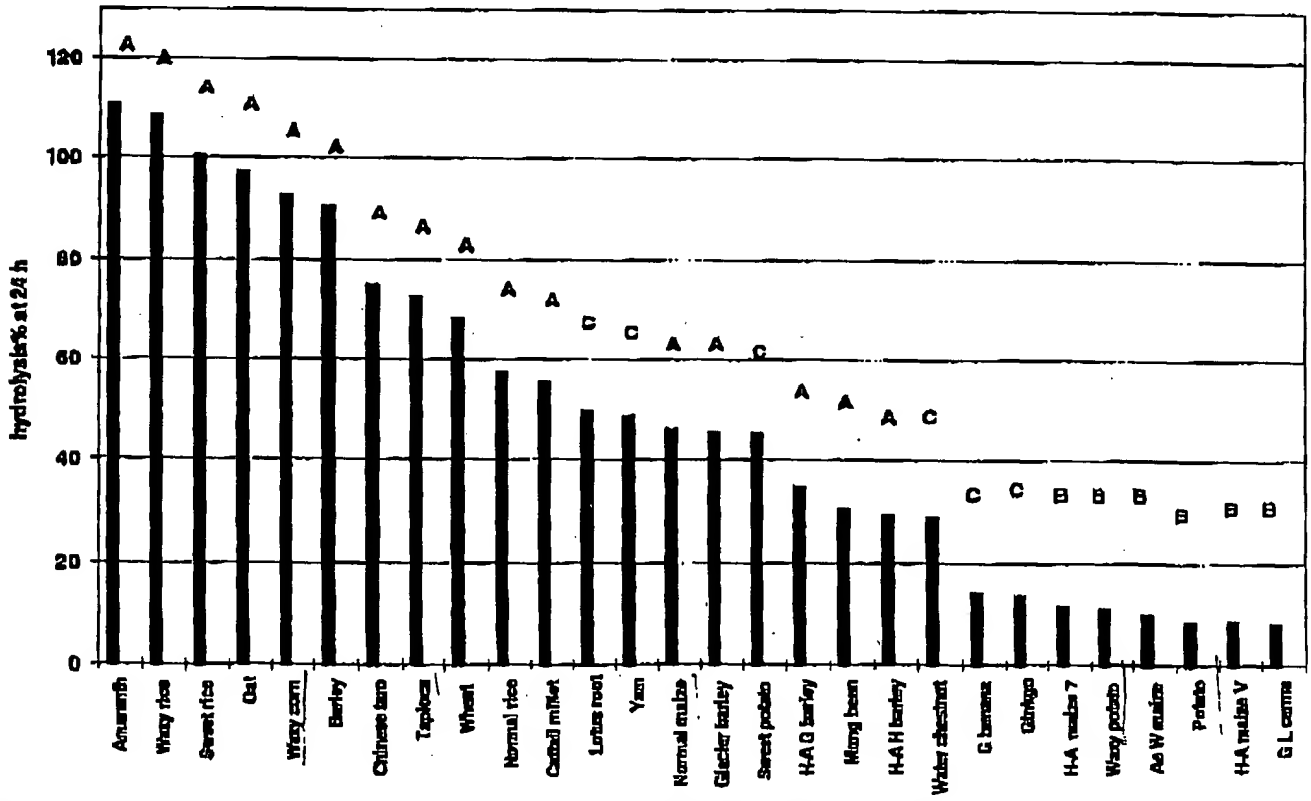

Fig. 1. Relative enzyme digestibility of selected granular starches (uncooked) of different crystalline structures.

The letter above each bar indicates the type of crystallinity of the starch. Porcine pancreatic α-amylase (120 U/20 mg starch in 9 mL phosphate buffer, pH 6.9) was used for the study, and the enzyme reaction was carried out at 37°C for 24 h. The percentage enzyme hydrolysis was determined by measuring the total carbohydrate content in the supernatant using phenol-sulfuric acid method.

Table 1. Amylopectin molecular weights and gyration radii of selected starches.[*1]

	Mw ($\times 10^5$)[*2]	Rz (nm)[*3]	ρ(g/mol/nm^3)[*4]
A-type starches			
Normal maize	4.9 (0.8)[*5]	312 (23)	16.1
Waxy maize	8.3 (0.2)	372 (11)	16.1
du wx maize	4.9 (0.5)	312 (13)	16.1
Normal rice	26.8 (2.9)	581 (41)	13.7
Waxy rice	56.8 (9.3)	782 (36)	11.9
Sweet rice	13.9 (1.0)	486 (5)	12.1
Normal wheat	3.1 (0.3)	302 (3)	11.3
Waxy wheat	5.2 (0.4)	328 (6)	14.7
Barley	1.3 (0.1)	201 (8)	16.0
Waxy barley	6.8 (0.1)	341 (3)	17.1
Cattail millet	2.7 (0.2)	278 (6)	12.6
Mung bean	3.8 (0.2)	312 (3)	12.5
Chinese taro	12.6 (3.6)	560 (15)	7.2
Tapioca	0.7 (0.1)	191 (25)	10.0
B-type starches			
ae wx maize	3.2 (0.2)	306 (8)	11.2
Amylomaize V	2.4 (0.0)	357 (24)	5.3
Amylomaize VII	1.7 (0.0)	389 (57)	2.9
Potato	1.7 (0.2)	356 (36)	3.8
Waxy potato	2.0 (0.2)	344 (37)	4.9
Green leaf canna	3.4 (2.2)	436 (85)	4.1
C-type starches			
Lotus root	1.5 (0.4)	280 (57)	6.8
Water chestnut	7.1 (1.5)	230 (25)	58.4
Green banana	1.9 (0.8)	286 (29)	8.1
Glycogen			
Cyanobacterial glycogen[*6]	0.2 (0.0)	55 (4)	99.2

[*1]Data were averages of at least two replicates. [*2]Weight-average molecular weight. [*3]z-Average radius of gyration. [*4]Density (ρ)=Mw/Rz^3. [*5]Standard deviation. [*6]Glycogen was isolated from *Synechocystis* sp. PCC6803 in our laboratory.

Structural differences between the large and the small starch granules: How are they synthesized ?

Barley and wheat starches are known to have two distinct groups of starch granules with different granule sizes: diameters of 15–32 μm for the barley large granule starch and 2–3 μm for the small granules (Figs. 3a, 3b and 3c). The large, A, granules display a disk shape, whereas the small, B, granules display spherical and irregular shapes. Both the large and small granule starches possess the A-type X-ray diffraction pattern. It has been reported by numerous researchers that the B granules display a higher gelatinization temperature (*ca.* 5°C at peak) than do the A granules (Table 2). Amylopectin molecular weights of the A and the B granules are 1.2×10^8 Da and 2.8×10^8 Da, respectively. Branch chain length distributions of the barley B starch granules showed more short chains (DP 6–12, 25.0%) and lesser B2 chains (DP 25–36, 14.7%) than did the A starch granules (22.6 and

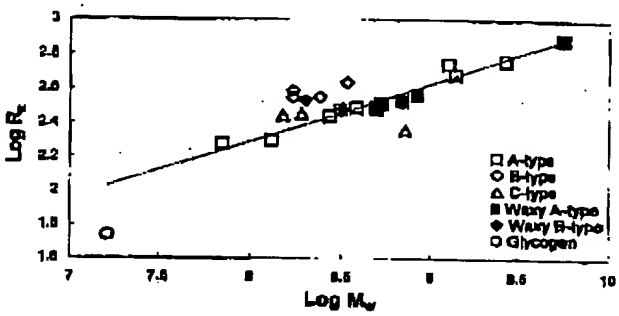

Fig. 2. Relationships between the weight-average molecular weight (Mw) and z-average radius of gyration (R_z) of amylopectins.

Data are plotted on Log–Log scale. The linear regression line on the graph comprises data of A crystalline-type amylopectin from Ref. 16).

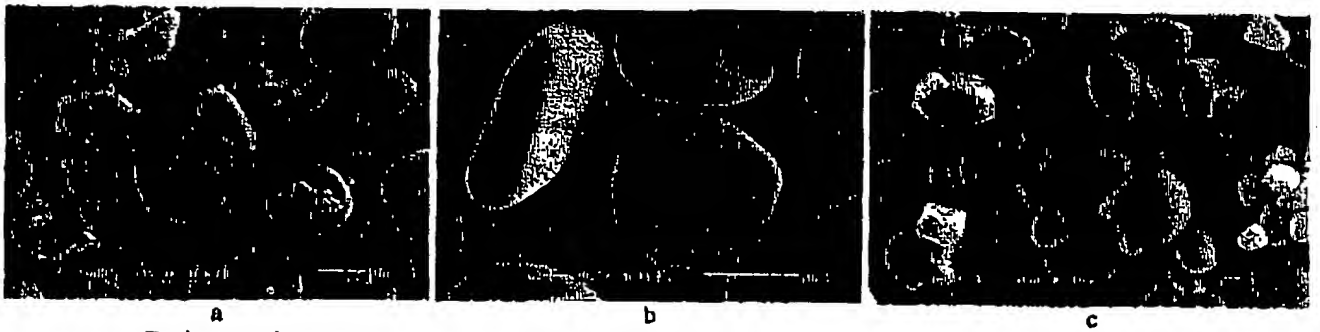

Fig. 3. Scanning electron micrographs of a) native, b) large granule and c) small granule barley starch.

Table 2. Gelatinization properties of barley starches.[*1]

Sample	Peak I[*2]				Peak II[*3]			
	T_o	T_p	T_c	ΔH (J/g)	T_o	T_p	T_c	ΔH (J/g)
Barley	57.9±0.3	62.6±0.1	67.9±0.2	12.6±0.3	94.1±0.2	99.5±0.5	103.3±0.3	0.9±0.1
Barley A type	57.0±0.1	61.5±0.4	66.4±0.4	12.2±0.7	94.1±0.4	99.5±0.4	103.4±0.7	0.9±0.2
Barley B type	58.2±0.2	66.3±0.4	73.6±0.6	12.7±1.2	94.4±1.2	101.9±1.2	109.2±1.7	2.1±0.2

[*1]T_o, T_p and T_c=onset, peak and conclusion temperatures (°C) of endotherm. ΔH=enthalpy change of gelatinization and melting of amylose-lipid complex. Values are mean±SD. [*2]Gelatinization. [*3]Melting of amylose-lipid complex.

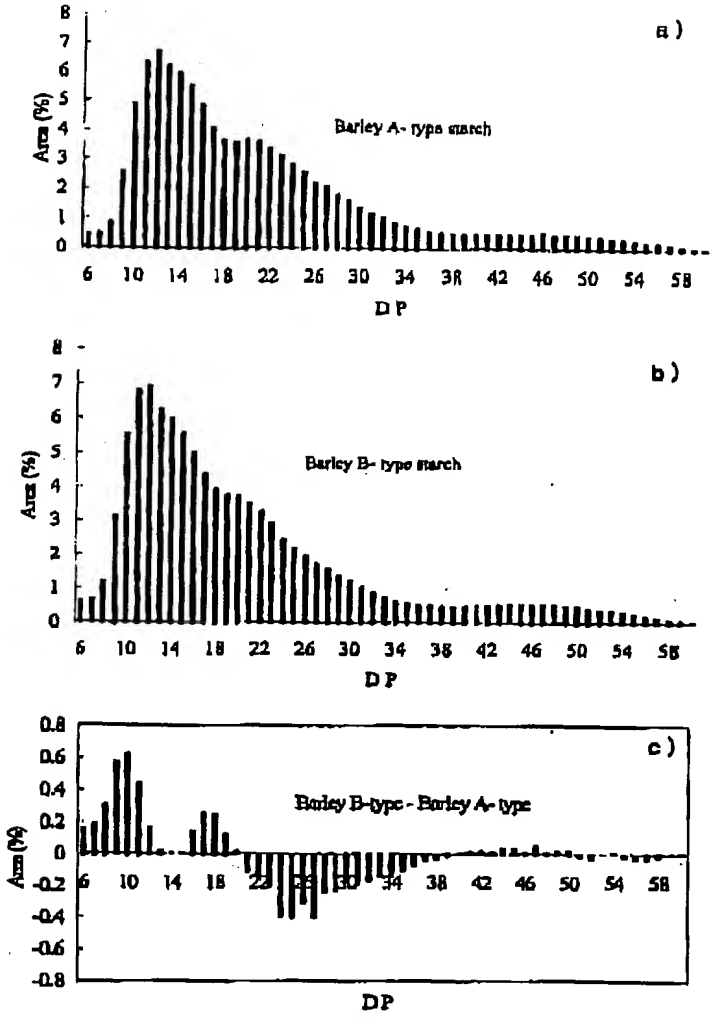

Fig. 4. High-performance anion-exchange chromatograms of debranched amylopectin of a) large (A) granule barley starch, b) small (B) granule barley starch, and c) differential histogram between B and A granules.

The debranched chains were separated by Dionex PA 100 column, treated with on-line amyloglucosidase, and detected by pulsed amperometric detector (HPAEC-ENZ-PAD).

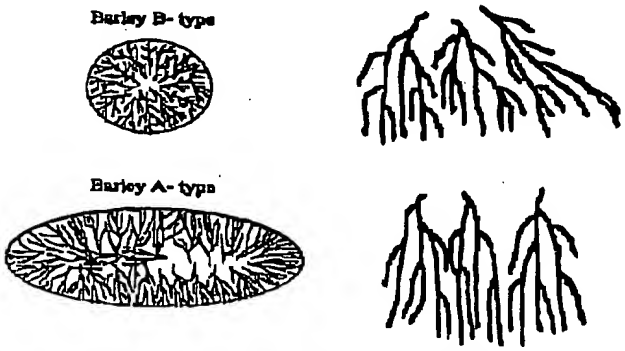

Fig. 5. Proposed granular and molecular structures of small and large granule barley starch.

The granular structures are constructed on the basis of polarized optical map of each starch granule proposed by the late Professor Dexter French (Ref. 23).

17.1%, respectively) (Fig. 4 and Table 3). The fact that the large (A) granules consist of more long branch chain and lesser short chains than the small (B) granules but display a lower gelatinization temperature contradicts the current understanding of the relationship between branch chain length and gelatinization temperature. For most starches investigated, a starch that possesses more short branch-chains and less long branch-chains displays a lower gelatinization temperature.[18-23] The histograms of branch chain length distributions of the two starches also showed that the A granule starch had a more distinct shoulder at DP 17–22. A differential histogram between the B granule starch and the A granule starch (Fig. 4c) showed that the B granule starch consisted of more branch chains of DP 6–12 and DP 16–21 but lesser chains of DP 22–38 than the A granule starch. Results from our previous studies indicate that a starch possessing lesser branch chains of DP 17–22 displays a lower gelatinization temperature.[21] This is attributed to the fact that the chain length of DP 17–22 is equivalent to the full length of the crystalline region of the amylopectin molecules. A starch that consists of fewer chains of the full length results in

Table 3. Branch chain-length distributions of amylopectins.

Sample	Peak DP		Distribution (%)				
	I	II	DP 6–9	DP 6–12	DP 13–24	DP 25–36	DP ≥37
Barley	12	46	4.9±0.1	23.7±0.7	50.1±1.2	16.4±0.2	9.8±2.02
Barley A-type	12	46	4.5±0.0	22.6±0.1	50.9±0.2	17.1±0.1	9.3±0.4
Barley B-type	12	44	5.7±0.0	25.0±0.1	50.9±0.2	14.7±0.2	9.5±0.2

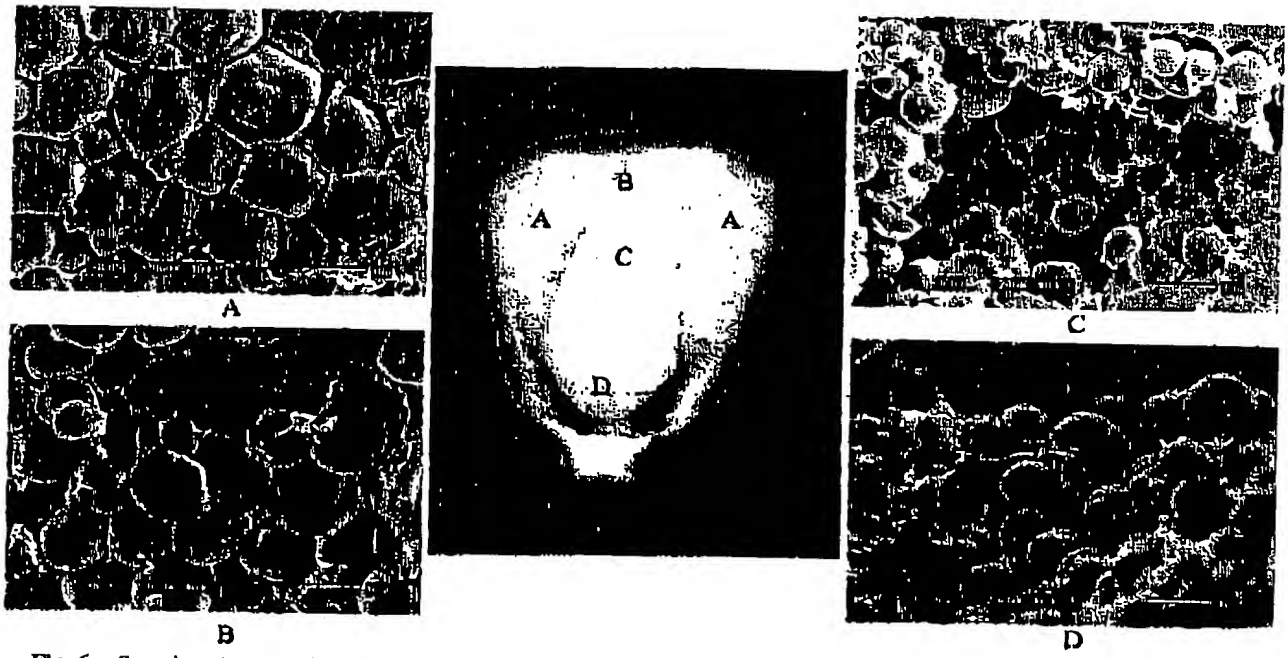

Fig. 6. Scanning electron micrographs of starch granules at different regions of a corn kernel.
Micrographs A–D show the starch granules located in each of the regions marked on the kernel.

defective crystals and lowers the gelatinzation temperature.

Based on polarized optical map studies, arrangements of starch molecules in the disk-shaped granules were proposed.[29] In a disk shaped granule, the majority of amylopectin molecules are oriented perpendicular to the flat surface of the granule. Thus, those molecules are parallel to one another. In a spherical granule, however, amylopectin molecules are oriented radially, which may give the molecules space to carry more short branch chains than those in the disk-shaped granule (Fig. 5).

Peng *et al.*[30] reported that fractionated large A granules of wheat starch consist of proteins that have molecular weights of 140 and 145 Da. These proteins are less or not found in the small B granule fraction. The authors designate the proteins as a starch branching enzyme 1c (SBE1c). The authors propose that the SBE1c is missing in the B granule and that prohibits the further development of the small, B granules to large, A granules. It will be intriguing to investigate if the SBE1c is responsible for synthesizing the larger proportion of DP 22–38 chains in large granules. It is also interesting to investigate if the larger proportion of DP 22–38 chains and a lesser number of short A and B1 chains make the amylopectin molecules fits better to the parallel arrangement of amylopectin molecules for the disk-shaped A granules.

What is the nature of pinholes on the surface of starch granules ?

Pinholes are commonly found on the granule surface of A-typed crystalline starches, such as maize, sorghum, but not on the B-typed crystalline starches, such as the potato. It has been postulated that pinholes are the results of enzyme degradation of starch. A recent study using scanning electron microscopy has shown that maize kernels, at a dormant state, display significant enzyme attack on starch, particularly at the region of the germ. SEM micrographs of starch granules at different region of a kernel are shown in Fig. 6. These results suggest that starch is hydrolyzed by enzymes to produce energy for seed germination even at the dormant state. The enzyme reaction is the most active around the region of the germ. Starch granules with pinholes may be isolated from the region around the germ of the seed.

REFERENCES

1) D. French: Organization of starch granules. in *Starch Chemistry and Technology*, R.L. Whistler, R.J. BeMiller and E.P. Paschall, eds., 2nd ed., Academic Press, Orlando, pp. 183–247 (1984).

2) J. Jane, A. Xu, M. Rodosovljivic and P.A. Seib: Location of amylose in normal starch granules. I. Susceptibility of amylose and amylopectin to cross-linking reagents. *Cereal Chem.*, 69, 405–409 (1992).

3) T. Kasemsuwan and J. Jane: Location of amylose in normal starch granules. II. Locations of phosphor-diesters and -monoesters revealed by ^{31}P-nmr. *Cereal Chem.*, 71, 282–287 (1994).
4) K. Denyer, B. Clarke, C. Hylton, H. Tatge and A.M. Smith: The elongation of amylose and amylopectin chains in isolated starch granules. *Plant J.*, 10, 1135–1143 (1996).
5) H. Gao, J. Imparl-Radosevich, H.P. Guan, P.L. Keeling, M.G. James and A.M. Myers: Identification of the soluble starch synthase activities of maize endosperm. *Plant Physiol.*, 120, 205–215 (1999).
6) C. Martin and A.M. Smith: Starch Biosynthesis. *Plant Cell*, 7, 971–985 (1995).
7) J. Preiss and M. Sivak: Starch synthesis in sinks and sources. in *Photoassimilate Distribution in Plants and Crops*, E. Zamski and A.A. Schaffer, eds., Marcel Dekker, New York, pp. 63–96 (1996).
8) Y. Nakamura: Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants: rice endosperm as a model tissue. *Plant Cell Physiol.*, 43, 718–725 (2002).
9) J. Jane and J.J. Shen: Internal structure of potato starch granule revealed by chemical gelatinization. *Carbohydr. Res.*, 247, 279–290 (1993).
10) D.D. Pan and J. Jane: Internal structure of normal maize starch granules revealed by chemical surface gelatinization. *Biomacromolecules*, 1, 126–132 (2000).
11) S. Hizukuri: Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydr. Res.*, 141, 295–306 (1985).
12) J. Jane, K.-S. Wong and A.E. McPherson: Branch-structure difference in starches of A- and B-type X-ray patterns revealed by their Naegeli dextrins. *Carbohydr. Res.*, 300, 219–227 (1997).
13) J.E. Fannon, R.J. Hauber and J.N. BeMiller: Surface pores of starch granules. *Cereal Chem.*, 69, 284–288 (1992).
14) C. Perera, Z. Lu, J. Sell and J. Jane: Comparison of physicochemical properties and structures of *sugary-2* corn starch with normal and waxy cultivars. *Cereal Chem.*, 78, 249–256 (2001).
15) S.-H. Yoo and J. Jane: Structural and physical characteristics of waxy and other wheat starches. *Carbohydr. Polym.*, 49, 297–305 (2002).
16) S.-H. Yoo and J. Jane: Molecular weights and gyration radii of amylopectins determined by high-performance size-exclusion chromatography equipped with multi-angle laser light scattering and refractive index detection. *Carbohydr. Polym.*, 49, 307–314 (2002).
17) Y. Takeda, C. Takeda, H. Mizukami and I. Hanashiro: Structures of large, medium and small starch granules of barley grain. *Carbohydr. Polym.*, 38, 109–114 (1999).
18) J. Jane, L. Shen, J. Chen, S. Lim, T. Kasemsuwan and W.K. Nip: Physical and chemical studies of taro starches and flours. *Cereal Chem.*, 69, 528–535 (1992).
19) Y.C. Shi and P.A. Seib: The structure of four waxy starches related to gelatinization and retrogradation. *Carbohydr. Res.*, 227, 131–145 (1992).
20) R.C. Yuan, D.B. Thompson and C.D. Boyer: Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three *wx*-containing genotypes in two inbred lines. *Cereal Chem.*, 70, 81–89 (1993).
21) J. Jane, Y.-Y. Chen, L.F. Lee, A.E. McPherson, K.-S. Wong, M. Radosavljevic and T. Kasemsuwan: Effects of amylopectin branch chain-length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chem.*, 52, 555–559 (1999).
22) N. Inouchi, Y. Sugimoto and H. Fuwa: Effects of amylopectin unit chains on the starch pasting characteristics. *Zymosc*, 7, 92–103 (2000).
23) D. French: Fine structure of starch and its relationship to the organization of starch granules. *J. Jpn. Soc. Starch Sci.*, 19, 4–25 (1972).
24) M. Peng, M. Gao, M. Baga, P. Hucl and R.N. Chibbar: Starch branch enzymes preferentially associated with A-type starch granules in wheat endosperm. *Plant Physiol.*, 124, 265–272 (2000).